European Journal of Soil Biology 123 (2024) 103690

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: www.elsevier.com/locate/ejsobi

Original article

Soil microbial resistance and resilience to drought under organic and conventional farming

Elena Kost [a,*], Dominika Kundel [b], Rafaela Feola Conz [a], Paul Mäder [b], Hans-Martin Krause [b], Johan Six [a], Jochen Mayer [c], Martin Hartmann [a]

[a] Institute of Agricultural Sciences, Department of Environmental Systems Science, ETH Zurich, Zurich, Switzerland
[b] Department of Soil Sciences, Research Institute of Organic Agriculture, Frick, Switzerland
[c] Department of Agroecology and Environment, Agroscope, Zurich, Switzerland

ARTICLE INFO

Handling editor: Dr S Geisen

Keywords:
Drought
DNA metabarcoding
Organic
Conventional
Soil prokaryotes and fungi
Wheat
Microbial resistance
Microbial resilience

ABSTRACT

The impacts of climate change, such as drought, can affect soil microbial communities. These communities are crucial for soil functioning and crop production. Organic and conventional cropping systems can promote distinct soil microbiomes and soil organic carbon contents, which might generate different capacities to mitigate drought effects on these cropping systems. A field-scale drought simulation was performed in long-term organically and conventionally managed cropping systems differing in fertilization and pesticide application. The soil microbiome was assessed during and after drought in bulk soil, rhizosphere, and roots of wheat. We found that drought reduced soil respiration and altered microbial community structures, affecting fungi in the bulk soil and rhizosphere more strongly than prokaryotes. Microbial communities associated with crops (i.e. rhizosphere and root) were more strongly influenced by drought compared to bulk soil communities. Drought legacy effects were observed in the bulk soil after harvesting and rewetting. The extent of the structural shifts in the soil microbiome in response to severe drought did not differ significantly between the organic and conventional cropping systems but each cropping system maintained a unique microbiome under drought. All cropping systems showed relative increases in potential plant growth-promoting genera under drought but some genera such as *Streptomyces*, *Rhizophagus, Actinomadura*, and *Aneurinibacillus* showed system-specific drought responses. This agricultural field study indicated that fungal communities might be less resistant to drought than prokaryotic communities in cropping systems and these effects get more pronounced in closer association with plants. Organic fertilization and the associated increase in soil organic carbon, or the reduction in pesticide application might not have the proposed ability to buffer severe drought stress on soil microbial taxonomic diversity. Yet, it remains to be elucidated whether the ability to maintain system-specific soil microbiomes also during drought translates into different functional capabilities to cope with the stress.

1. Introduction

Drought events are projected to increase due to climate change in certain regions of the globe [1], which can threaten crop yield and health [2]. However, drought stress not only affects plants but also soil microbiomes and their functions [3,4], showing possible legacy effects [5]. Soil microbes have evolved different mechanisms to adapt to drought, including osmolyte accumulation [6], production of exopolymeric substances [7], thickening of cell walls [8], and dormancy [9]. Soil respiration, an indicator of microbial activity, and microbial abundance often decrease under water limitation [10,11]. Many studies report an effect of drought on microbial community composition, with bacteria being usually more affected by water limitation than fungi [3,4]. Fungi have thick cell walls, osmolytes, melanin, and a large hyphal network [12,13], which can improve their drought tolerance. However, bacteria can become dormant during droughts, mostly live in small pores and microaggregates that dry out slowly and were shown to form under reduced precipitation [14], thereby also being able to tolerate drought events [6,9]. Slow-growing oligotrophic bacteria that can maintain growth under nutrient-poor conditions are considered to be better adapted to water-limited conditions compared to copiotrophs that thrive under nutrient-rich and well-watered conditions [15].

* Corresponding author. Universitaetstrasse 2, 8092, Zürich, Switzerland.
E-mail address: elena.kost@usys.ethz.ch (E. Kost).

https://doi.org/10.1016/j.ejsobi.2024.103690
Received 5 September 2024; Received in revised form 21 October 2024; Accepted 23 October 2024
Available online 5 November 2024

The soil microbiome is essential for climate regulation, nutrient cycling, plant growth promotion and stress tolerance, disease and pathogen control, and pollutant degradation [16]. Some microbes, referred to as plant-growth-promoting, can improve plant drought tolerance and potentially alleviate negative impacts of drought on crops by, for example, increasing plant osmolyte, abscisic acid, or auxin concentrations, decreasing plant ethylene concentration, or producing exopolymeric substances [17–19]. Plant roots associate with microbial communities that are located in the soil around roots (rhizosphere), on roots (rhizoplane), or inside roots (endosphere) [20]. Recent studies showed that microbial communities in the rhizosphere and endosphere react more strongly to drought compared to bulk soil communities [3,21], which might be related to the effects of drought-affected plant rhizodeposition on the associated microbes [22,23].

Many studies assessing the effects of drought on soil microbes have been conducted in grasslands, greenhouses, or in only one type of cropping system. However, it has been reported that different cropping systems, under organic or conventional practices, can promote distinct soil microbiomes [24], which might differ in their ability to respond to drought. More resilient and resistant microbial communities are suggested to have greater abilities to maintain soil functions under stress such as drought [25]. Resistance and resilience are defined as the ability to tolerate and recover from disturbances, respectively [25]. Resistance and resilience can occur at the structural and functional level, whereas it has been shown that a lack of structural resistance or resilience often translates into a lack of functional resistance or resilience [26]. In the context of this study, we defined resistance as the lack of shifts in microbial community composition upon drought and resilience as the return of the microbial community composition to the status in rainfed control conditions.

Organic, biodynamic, and conventional cropping systems differ in fertilization, pesticide application, and crop rotation. Since no synthetic pesticides and mineral fertilizers are applied in organic and biodynamic cropping systems, fertilization is done with green manure, stacked or composted manure, slurry, and by incorporating legumes into the crop rotation. Systems receiving organic amendments generally show higher soil microbial biomass, enzyme activity, microbial diversity, and activity [24,27,28]. Moreover, higher soil organic carbon (SOC) contents have been reported in organic cropping systems due to manure application and higher SOC contents were observed in systems receiving composted manure versus stacked manure [29]. Increased SOC is considered to increase soil aggregation, porosity, and water retention [30]. Thus, higher SOC contents (i.e., improved soil structure and moisture retention) and enhanced microbial diversity and abundance might have the potential to increase microbial resistance and resilience towards drought [31,32].

Previous studies comparing microbial resistance and resilience to drought in organically and minerally fertilized treatments were often conducted in greenhouses and sometimes even without the cultivation of plants [33–37]. Such pot experiments apply organic fertilizers only over a short time period and under controlled conditions, which might not translate well to the field-scale agronomic context characterized by frequent disturbances due to management interventions and long-term organic fertilization resulting in lasting shifts in SOC stocks. Therefore, field-scale studies in cropping systems that have been managed organically or conventionally over longer timescales are required to derive more realistic data. To close this gap, Kundel et al., 2020 [38] performed a sheltering experiment to study the contrasting response of soil prokaryotes and fungi to drought in a long-term cropping system trial called the DOK experiment, which compares different organic and conventional cropping systems since 1978 [38,39]. Previous studies have shown that these cropping systems in the DOK trial differ, among other factors, in SOC content and microbial community structure [24,29]. However, the study by Kundel et al., 2020 [38] failed to successfully induce a strong water gradient between the rainfall reduction and control plots. Further, they compared only the biodynamic to the minerally fertilized conventionally managed systems, which consequently did not allow disentangling the effects of organic fertilization from the effects of pesticide application since these factors both differ between these systems. Thus, we still have an incomplete understanding of the extent to which differences in fertilization or pesticide application in the organic and conventional cropping systems diverge in their capacity to increase microbial resistance and resilience to drought under realistic field conditions [40].

To address these issues, this study aimed to induce a severe summer drought by applying a complete rainout sheltering in the biodynamic, mineral conventional, and mixed conventional systems of the DOK trial, and measuring the impact on prokaryotes and fungi in bulk soil, rhizosphere, and root. Sampling of the winter wheat took place three times during the drought period and twice after rewetting to assess the microbial resistance and resilience, respectively. Based on the current literature described above, we hypothesized that (i) the effects of drought will be stronger on prokaryotic than on fungal community structure, and (ii) this drought effect will get stronger in closer association with the plant (e.g., stronger in root than rhizosphere than bulk soil). We further hypothesized that the microbial communities in the different compartments (i.e., bulk soil, rhizosphere, and root) will show contrasting structural (iii) resistance and (iv) resilience towards severe drought stress depending on the cropping system, showing increasing resistance and resilience with increasing SOC contents in the following order: a conventionally managed system exclusively receiving mineral fertilization (low SOC), an integrated conventional system receiving a combination of mineral fertilizer, stacked manure, and slurry (intermediate SOC), and a biodynamic system fertilized with composted manure, and slurry (high SOC).

2. Methods

2.1. Experimental design

An on-field drought simulation experiment was conducted in the DOK long-term trial, which has been described in more detail by Krause et al. (2022) [29]. Briefly, the field site is located on a Haplic Luvisol in Therwil, Switzerland (47°30′9.48"N, 7°32′22.02"E). The trial compares five different organic and conventional cropping systems differing in fertilization and pesticide management since 1978. The average annual precipitation at this field site is 840 mm and the mean annual temperature is currently around 11 °C [29].

Rainout shelters, described by Malisch et al. (2016) [41], were established with rain gutters in mid-November 2021 in three cropping systems (6 m × 4 m x 2.4 m; Fig. 1). The shelters were placed on one side of the plots and the corresponding rainfed controls were established on the other side (Fig. 1). To avoid legacy effects from the previous partial sheltering study [38], rainout shelters were installed in different experimental blocks. Three out of the five cropping systems included in the DOK trial were selected based on the most contrasting biological, physical, and chemical soil properties as found in previous studies [24,39]. The biodynamically managed system (subsequently referred to as BIODYN) is fertilized with composted farmyard manure and slurry, receiving biodynamic preparations, no chemical pesticides, and managed according to the guidelines of Demeter Schweiz (2019) [42]. The weed control is done mechanically. The other two systems were managed conventionally, one mixed system receiving a combination of stacked farmyard manure, slurry, and mineral fertilizers (CONFYM) and one exclusively minerally fertilized system (CONMIN). The conventional systems were treated with herbicides, fungicides, insecticides, and synthetic plant growth regulators (chlormequat chloride and trinexapac-ethyl) according to Swiss regulations [43]. The manure-based systems (BIODYN, CONFYM) represent mixed crop-livestock systems and received organic amendments corresponding to a stocking density of 1.4 livestock units per hectare and year. The cropping systems do not differ in their crop rotation, tillage practice, and

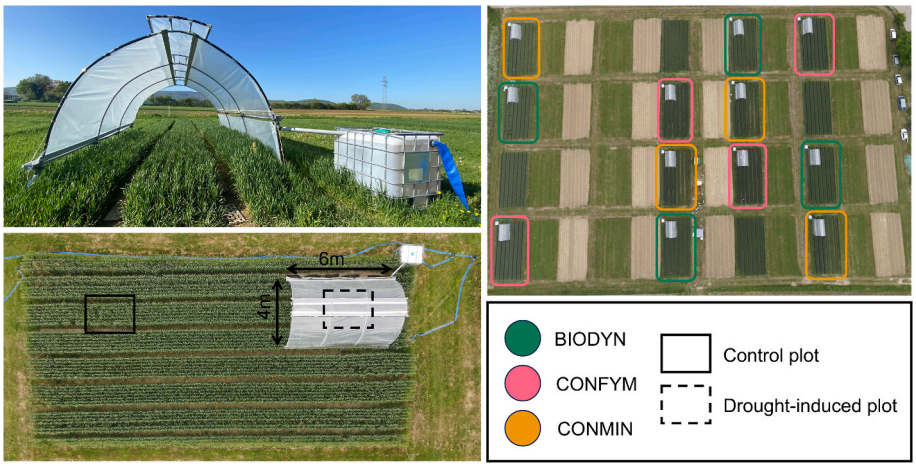

Fig. 1. Experimental design of the on-field rainout sheltering experiment in the DOK long-term field trial across three different cropping systems (biodynamic - BIODYN, conventional mixed - CONFYM, and conventional - CONMIN) with winter wheat.

depth to which all soil preparations were done (Table S1). All treatments were replicated four times (3 cropping systems x 2 water regimes x 4 replicates). Winter wheat (*Triticum aestivum* var. Wiwa) was sown mid-October 2021. A detailed timeline of all on-field interventions during the experiment is provided in Table S1. In brief, plots were irrigated by precipitation until shelter installation. Shelters were installed in November 2021 and sheltered plots were irrigated during winter 2022 using watering cans with a total of 55 mm of rainfall equivalent of either precipitation or tap water until beginning March. Since the field side is located on a Halpic Luvisol with a high soil water retention, rainfall was already reduced in November. The soil water content was monitored as mentioned below with sensors to ensure that soil water content was not reduced in the sheltered plots compared to the control in winter. The control plots received around 193 mm of rainfall from mid-November 2021 to April 2022, compared to a long-term average of around 217 mm. The sheltered plots were then completely deprived from water between 1 April and July 14, 2022. Hence, precipitation was reduced by 72 % during the winter months and completely removed from April to mid-July. A soil water content of around 10 % was targeted since this was described in previous studies as severe drought stress [33,44,45], which is predicted to increase due to climate change [46]. After shelter removal and harvesting of the wheat, a rewetting was done on both sheltered and control plots with 36 mm of tap water, and the plots were exposed to rainfed conditions from then on. The entire experiment lasted from mid-November 2021 to mid-September 2022.

Soil moisture and temperature were monitored in one replication in each of the six experimental treatments at two depths (5 and 20 cm) by time domain reflectometry soil sensors (TDR sensors; METER Group, Pullman, WA, USA) and in all replicated plots by TOMST sensors (TOMST, Prague, Czech Republic) down to 15 cm depth. Gravimetric soil water content (GWC) in 0–15 cm was measured at all sampling campaigns. Air temperature was measured on soil and vegetation level by TOMST and HOBO (EnviroMonitors, Arundel, United Kingdom) sensors, respectively. The latter also measured air humidity. Photosynthetic active radiation (PAR) was measured by PAR Photon Flux Sensors (METER Group) on vegetation level. The HOBO and PAR sensors were installed in the same six plots as the TDR sensors.

2.2. Sampling

Sampling events took place at five timepoints. The first three sampling campaigns were during the wheat growing and drought period at (i) stem elongation, (ii) flowering, and (iii) grain ripening. Plant stage, plant height, aboveground plant, and ear biomass were recorded on an area of 0.042 m^2 (three wheat rows of 17.5 cm × 8 cm) at each timepoint. Since seed density was 400 grains m^{-2} approximately 16–17 plants were harvested on this area. Bulk soil samples were taken between the rows with a soil corer (diameter of 5 cm) down to 15 cm (n = 3). Wheat roots with the surrounding soil core were sampled for rhizosphere and root microbial analysis within rows using a soil auger (diameter of 8 cm) to a depth of 15 cm (n = 3) and loose soil was manually removed by shaking. At the fourth and fifth sampling campaigns (iv) one week and (v) eleven weeks after harvesting and rewetting, respectively, bulk soil was sampled down to 15 cm (n = 3). All bulk soil samples were homogenized and sieved to 5 mm. Bulk soil and root samples were stored at −20 °C until further processing.

2.3. Soil respiration

In-situ soil respiration was measured as described in more detail by Barthel et al. (2022) [47]. Briefly, soil respiration was measured weekly during the wheat vegetation period using the non-steady-state, static chamber method with chambers of 30 cm diameter and 30 cm height. Chambers were installed in the field early January. Importantly, wheat plants and weeds were removed throughout the seasons within the chambers to reduce plant respiration. For the gas flux measurements, chambers were closed for 1 h, and four air samples were collected at 20-min intervals. Temperature was measured at a metrological station on the field. Carbon dioxide (CO_2) and methane (CH_4) concentrations in samples were measured by gas chromatography (456-GC; Scion Instruments, Goes, The Netherlands) using standards covering the expected range of concentrations. The coefficient of determination (R^2) of the linear regression of $\frac{\Delta n}{\Delta t}$ (i.e., the rate of change in concentration in mol s^{-1}) from flux data was higher than 0.95 for 94 % of the CO_2 data and 38 % of the CH_4 data. The low coefficient of determination of the CH_4 data indicates that there is no strong methane flux.

2.4. Plant and soil measurements

Plant height, aboveground plant, and ear fresh weights were recorded in the field. The dry biomass was assessed after drying samples at 40 °C to constant weight. Ground plant samples were digested at 120 °C for 90 min with 15 mL of nitric acid (65 % HNO_3) followed another 90 min with 3 mL of hydrogen peroxide (30 % H_2O_2) at 120 °C. Digests were analyzed by ICP-OES. The soil was dried at 105 °C until constant weight to assess the gravimetric water content. The pH was assessed in a soil suspension with deionized water (1:2.5, w/v). Total soil carbon (C) and nitrogen (N) were determined on dried samples with the Dumas method. Magnesium was measured by flame atomic absorption spectroscopy in $CaCl_2$ extracts (1:10, w/v). Plant-available soil phosphorus and

potassium were measured photometrically and by flame atomic emission in CO_2-saturated water extract (1:2.5, w/v), respectively.

2.5. Rhizosphere and root separation

After thawing, roots were cut from the sampled rootstocks into a 30 mL buffer solution (6.75 g KH_2PO_4 and 8.75 g K_2HPO_4 in 1000 mL deionized water, adding 200 μL Tween 20 after autoclaving), vortexed for 2 min, and roots were separated into bags. Root samples were freeze-dried and ground with the FastPrep-24™ 5G (MP Biomedical, Irvine, CA, USA). The remaining buffer solution containing the rhizosphere soil was sieved through a 2 mm mesh to remove residual root debris, centrifuged for 10 min at 4 °C with 4700×*g*, and decanted. The resulting pellet was stored at −20 °C.

2.6. Nucleic acid extraction

The DNeasy ® PowerSoil ® Pro Kit (Qiagen, Hilden, Germany) was used to extract DNA on the QIAcube Connect instrument (Qiagen) according to the manufacturer's recommendation from 0.25 g homogenized rhizosphere and bulk soil, as well as from 0.04 g homogenized and lyophilized roots. Blanks were included in every run and yielded no PCR amplification. DNA quality and quantity were assessed via UV/VIS spectrophotometry on a QIAxpert instrument (Qiagen) and normalized to 10 ng μL^{-1}.

2.7. Metabarcoding

The bacterial and archaeal (hereafter termed prokaryotic) 16S rRNA gene (V3-V4 region) and the fungal ribosomal internal transcribed spacer (ITS2 region) were PCR amplified with primers 341F/806R and 5.85-Fung/ITS4-Fung using the conditions described in Table S2. For root samples mPNA/pPNA clamps (PNA BIO, Newbury Park, CA, USA) were used to inhibit the amplification of organelle DNA with the 16S rRNA gene primers (Table S2). PCR products were generated in technical triplicates, which were pooled in equal volumes and sent to the Functional Genomics Center Zurich (FGCZ, Zurich, Switzerland) for indexing PCR. Indexed PCR products were purified, quantified, and pooled in equimolar ratios before pre-sequencing on the Illumina MiniSeq platform (Illumina Inc., San Diego, CA, United States) to inform library re-pooling for optimal equimolarity across samples. Final sequencing was conducted using the v3 chemistry (PE300) on the Illumina MiSeq platform (Illumina Inc.).

The sequence data were quality filtered, delineated into amplicon sequence variants (ASVs), and taxonomically classified against SILVA v138.1 for prokaryotes [48] and UNITE v9.0 for fungi [49] using a customized pipeline as described previously [50]. In brief, the pipeline included primer trimming, PhiX filtering, paired-end read mering, quality filtering, dereplication, ASV delineation, chimera removal, target verification, read mapping and taxonomic classification. The total read number was 14 073 236 (53 920 ± 8969 per sample) for 16S rRNA gene and 11 725 012 (44 582 ± 16 984 per sample) for ITS2 sequences. Sequences were assigned to 42 108 and 3801 ASVs after quality control and taxonomic assignment for prokaryotes and fungi, respectively. Prokaryotic ASVs were classified into copiotrophic and oligotrophic lifestyles based on rrn gene copy numbers on the lowest taxonomic rank classified using rrnDB v5.8 [51] and applying the thresholds of ≥5 for copiotrophs and <5 for oligotrophs [52].

2.8. Quantitative real-time PCR

Prokaryotic and fungal abundance in bulk soil was measured with a SYBR® Green-based quantitative PCR (qPCR) approach targeting the 16S (primer specific for prokaryotes) or 18S (primer specific for fungi) rRNA gene as described by Jaeger et al. (2023) [44], including a test for potential amplification inhibition, generation of standard curves from purified PCR products of different concentrations, and qPCR amplification of the samples in technical triplicates. The PCR conditions are described in Table S2. Amplification efficiencies ranged between 92 and 100 % for (16S) and 75–80 % (18S) with an R^2 of ≥0.95 (16S) and ≥0.99 (18S).

2.9. Statistics

All statistical analyses were performed with R Version v4.3.1 [53] and R Studio Version 2023.06.2 + 561 [54]. P- and q-values <0.05 were considered significant unless mentioned otherwise. In case of small statistical effects, a higher false discovery rate was allowed (i.e., q < 0.1). All permutation-based tests were performed with 9999 permutations. All data were visualized with the R package *tidyverse* version v2.0.0 [55].

Effects of water regime, cropping system, and their interaction on GWC, plant parameters (height, biomass, plant nutrients), and cumulative CO_2 (log-transformed) were analyzed by a two-way ANOVA for each sampling date separately when requirements of homogeneity of variance and normal distribution of the residuals were fulfilled. Posthoc tests were performed using Tukey adjustments for multiple testing. In case the normal distribution of the residuals was not fulfilled, effects of the (i) water regime, cropping system, and their interaction or (ii) water regime, cropping system, sampling date, and their interactions on (i) 16S and 18S rRNA gene copy numbers, the ratio of copiotrophs to oligotrophs for each sampling date, and on (ii) soil chemical properties for all sampling dates combined were analyzed with a univariate permutational analysis of variance (PERMANOVA) [56] and permutational analysis of multivariate dispersion (PERMDISP) [57] using the *adonis2* and *betadisper* functions in the package *vegan* v2.6.4 [58]. Pairwise comparisons were done with the function *pairwise.perm.manova* in the RVAideMemoire package v0.9-83 [59]. After transforming the logger and flux data in case of non-normality or heteroscedasticity (e.g., soil respiration, methane emission, soil moisture, humidity, PAR, soil and air temperature) using *bestNormalize* v1.9.0 [60], they were analyzed with one-way (in case of logger data, which were only installed in few plots) or two-way ANOVA including adjusting for repeated measures. Subsequent post hoc tests were performed using multiple comparisons of least-square means, and was adjusted for multiple testing using Tukey with the packages *emmeans* v1.10.1 and *multcomp* v1.4.25 [61].

Rarefaction curves (Fig. S1) were calculated to inspect the sequencing depth using the *rarecurve* function in *vegan*. To account for differences in sequencing depth across samples [62], ASV tables were 100-fold iteratively subsampled to the minimal read number using the *rrarefy* function in *vegan,* and the average α and β-diversity metrics were calculated based on the 100 subsampled matrices. The Shannon diversity index was calculated using the function *diversity* in *vegan*. The β-diversity was assessed based on Bray-Curtis dissimilarities implemented by the function *vegdist* in *vegan*. The effects of water regime, cropping system, sampling date, and their interactions on α- and β-diversity were assessed by univariate and multivariate PERMANOVA and PERMDISP, respectively. Unconstrained ordinations were performed using principal coordinate analysis (PCoA) with the *cmdscale* function in *vegan*. Constrained ordinations were performed using canonical analysis of principal coordinates (CAP) [63] with the *CAPdiscrim* function in the *BiodiversityR* package v2.15.2 [64]. The read counts of each ASV assigned to the same taxonomic group were aggregated across the taxonomic hierarchy and used to test the individual response of taxonomic groups to water regime, cropping system, sampling date, and their interactions using PERMANOVA followed by adjustments for multiple testing using the *qvalue* function in *qvalue* v2.32.0 [65]. Data were z-transformed for visualization of the differences in relative abundances between all treatments using the *scale* function in R. Genera responding significantly were displayed using iToL v6.8.1 [66], using taxonomic trees built from the taxonomy table using the *taxa2dist* function in *vegan* and the *hclust* function in *ade4* package v1.7-22 [67].

3. Results

3.1. Successful implementation of severe drought

The GWC was significantly reduced during the drought period in sheltered plots compared to the control from on average 26 % to 9 % (Fig. 2), supported by the continuous TOMST and TDR sensor measurements (Fig. S2). After rewetting, soil moisture increased and showed no significant difference between the water regimes at the second sampling after rewetting (Fig. 2). No significant ($p > 0.05$) interaction was observed between soil water reduction in drought-induced plots and cropping systems at any of the sampling timepoints. Soil temperature below the rainout shelter increased by 1.6 ± 0.4 °C at 5 cm depth and 1.1 ± 0.2 °C at 20 cm depth compared to the control (Fig. S3). Air temperature slightly increased by 1.2 ± 0.1 °C and 0.4 ± 0.1 °C below the rainout shelter compared to controls assessed at 15 cm above the ground (Fig. S4) and wheat vegetation level ($F = 18.4$, $p = 0.013$; data not shown), respectively. Humidity was not influenced by the sheltering ($F = 0.1$, $p = 0.782$; data not shown), while the mean PAR was reduced by 28 ± 2 % due to sheltering (Fig. S4).

3.2. Drought reduces plant nutrients and growth

All plant nutrients in the shoot and ear (e.g., total nitrogen, carbon, potassium, magnesium, and phosphorus) were affected by drought but not at all sampling dates (Fig. S5). The content of nitrogen in the shoot (29 ± 1 %) and ear (9 ± 5 %), carbon in the ear (1 ± 0 %), potassium in the shoot (31 ± 7 %) and ear (13 ± 5 %), magnesium in the ear (9 ± 7 %), and phosphorus in the shoot (44 ± 9 %) and ear (18 ± 8 %) were significantly reduced under drought conditions mostly across all cropping systems. However, the shoot nitrogen content at ripening and ear phosphorus content at flowering was only significantly higher in the control in CONFYM. Shoot phosphorus contents at stem elongation and ear potassium contents at flowering were all significantly higher in the control but the highest difference was found in CONFYM, followed by BIODYN and CONMIN. The significant interactions between the water regime and cropping system on the carbon content in the shoot and ear indicated by ANOVA were not confirmed by the subsequent post hoc test. The magnesium content in the shoot was first increased but then decreased at ripening in the control compared to the drought. The carbon content in the shoot was decreased in the control at stem elongation and flowering. All plant nutrients were affected by the cropping system, except magnesium and phosphorus only at one timepoint.

At the first sampling (i.e., stem elongation), wheat plants below the rainout shelter were already at Zadok growth stage 32, whereas the plants in the rainfed control were only at 31. At the second sampling (i.e., flowering), sheltered and rainfed plants all completed flowering (Zadok stage 68), although the flowering started a few days earlier below the shelters. At the last sampling (i.e., ripening) plants in the drought-induced plots were at Zadok stage 93, while they were at Zadok stage 92 in the control plots. Plant height was significantly increased by 28 ± 7 % below the rainout shelters at stem elongation (Fig. S6). At flowering and ripening, plant height was lower by 7 ± 4 % and 9 ± 5 %, respectively, below the shelters compared to the control. However, drought and cropping systems showed an interactive effect, which was reflected by larger differences between sheltered and control plots in the two conventional systems (CONFYM, CONMIN) as compared to the BIODYN system (Fig. S6). Drought significantly reduced the total fresh weight at flowering and ripening by 27 ± 11 % and 33 ± 19 %, respectively (Fig. S6). The sheltering significantly increased the total dry biomass of wheat at stem elongation while no differences between the water regimes were observed at flowering and ripening (Fig. S6).

3.3. Drought reduces soil nutrients

Plant-available phosphorus and potassium concentrations in the soil were significantly influenced by drought (Table S3), showing an increase of 16 ± 5 % for phosphorus and 35 ± 14 % for potassium during drought in the sheltered plots. The effect of drought on potassium was dependent on the cropping system and increased under drought in all systems but most strongly in the conventional system. This increase in available potassium and phosphorus in the drought plots disappeared after the rewetting (Table S4). After the rewetting, the potassium was higher in BIODYN by an average of 23 ± 11 % compared to the

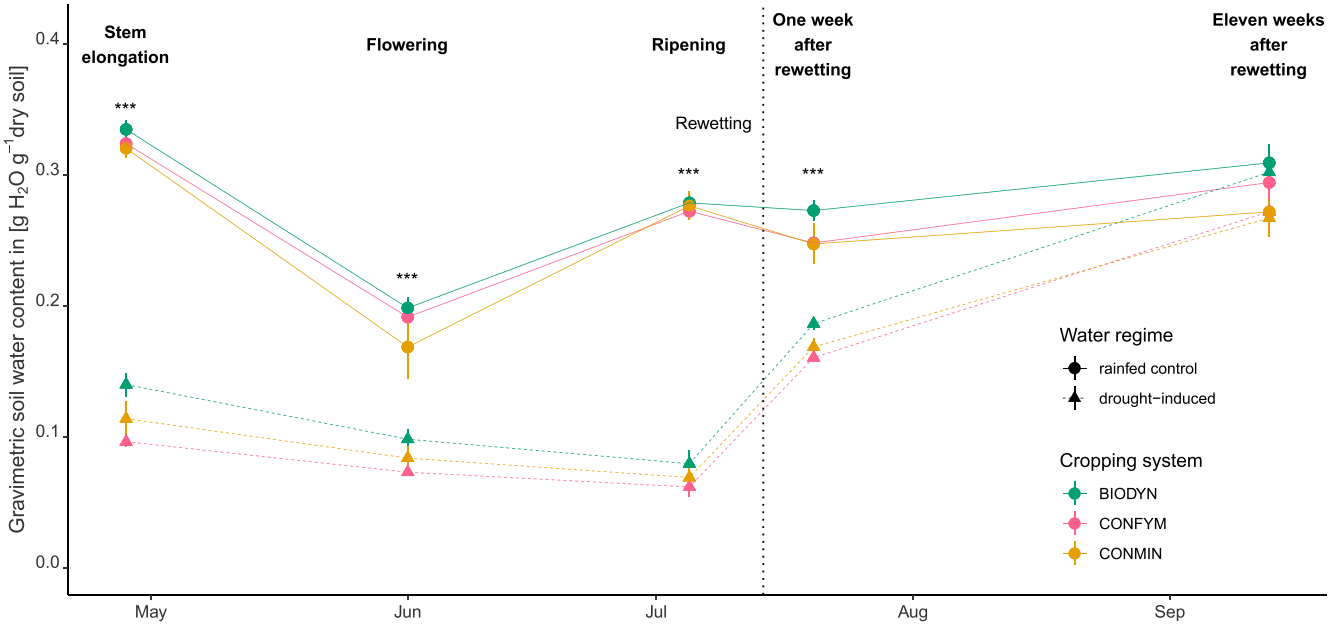

Fig. 2. Gravimetric water content (GWC) for each cropping system in drought-induced and rainfed control plots across the five sampling points to 15 cm depth. Asterisks indicate significant (ANOVA, $p < 0.001$, $n = 12$) differences between drought and control plots. Means and standard errors are shown.

conventional systems. While plant-available phosphorus was mainly increased in CONFYM under drought, it was higher in both CONFYM and BIODYN compared to COMIN after rewetting. The plant-available magnesium was increased in CONMIN and CONFYM over the whole period. The other soil chemical properties (i.e., total C and N, plant-available magnesium, pH) showed no significant differences between the water regimes. Total C and N, pH were significantly increased in the biodynamic system compared to the conventional systems during drought and after the rewetting (Tables S3 and 4).

3.4. Drought reduces soil respiration

Drought significantly ($p < 0.001$) reduced *in-situ* soil respiration by an average of 25 ± 8 % over the whole drought period, but with strong fluctuations over time ($p < 0.001$; Fig. 3a). Although a small significant interactive effect of the water regime and cropping system was found by the ANOVA ($p < 0.05$), this could not be confirmed by the following post hoc analysis. Agricultural management significantly influenced soil respiration across both water regimes, having the lowest soil respiration in BIODYN compared to the conventional systems ($p < 0.01$). Additionally, a drought effect on the cumulative CO_2 flux was reported, showing no effect of cropping system or interactive effect of water regime and cropping system (Fig. 3b). On average methane uptake was recorded, but with high variability between replicates, nevertheless, showing an increased methane sink by 23 ± 35 % under drought compared to the rainfed controls ($p < 0.001$). A significantly lower methane uptake was recorded for CONMIN when compared to BIODYN and CONFYM ($p < 0.05$).

3.5. No drought effect on microbial abundance

Microbial abundance in the bulk soil approximated by the quantification of prokaryotic 16S and fungal 18S rRNA gene copy numbers were not significantly affected by drought (Fig. S7). A significant decrease of the fungi to prokaryotes (F/P) ratio was found below the shelters at the first and an increased F/P ratio was observed at the last timepoint after rewetting, respectively. There was a significantly lower F/P ratio in BIODYN compared to the conventional systems, independent of the water regime (Fig. S7).

3.6. Drought alters microbial community composition

Since the compartments (i.e., bulk soil, rhizosphere, and root) showed an overriding effect ($p < 0.001$) on microbial communities, compartment data were analyzed separately. Differences in relative abundances of major taxonomic groups between compartments are illustrated in Fig. S8.

Prokaryotic α-diversity (assessed as Shannon index) was not influenced by drought, whereas fungal α-diversity significantly decreased in the rhizosphere and increased in the root during drought compared to the control (Table S5). No interaction between drought response and cropping system on α-diversity was found for fungi or prokaryotes.

Major shifts in the prokaryotic and fungal community composition between the cropping systems were detected in all compartments (Table 1, Fig. S9, Fig. 4). This was followed by a subordinate effect of drought on microbial community composition. The cropping system explained between 9.9 and 30.7 % of the variance and decreased from bulk soil (22.5–30.7 % of the variance) to rhizosphere (18.1–29.8 %), and root (9.9–20.0 %) (Table 1). In contrast, the effect of drought increased from bulk soil (1.5–5.7 %), to rhizosphere (3.1–7.8 %), and root (6.8–11.5 %) and was significant for all comparisons except the prokaryotic β-diversity in the bulk soil (Table 1). This drought effect was largely independent of the cropping system, showing a significant interaction between the water regime and cropping system only for the prokaryotes in the roots (Table 1). The plant development (i.e., sampling date) explained 1.2–6.4 % of the variation in β-diversity and significantly affected fungi in all compartments and prokaryotes in the root only (Table 1). The effect of drought depended on the sampling date indicated by a significant interaction in the rhizosphere and root for fungi, and in the root for prokaryotes (Table 1). An increased

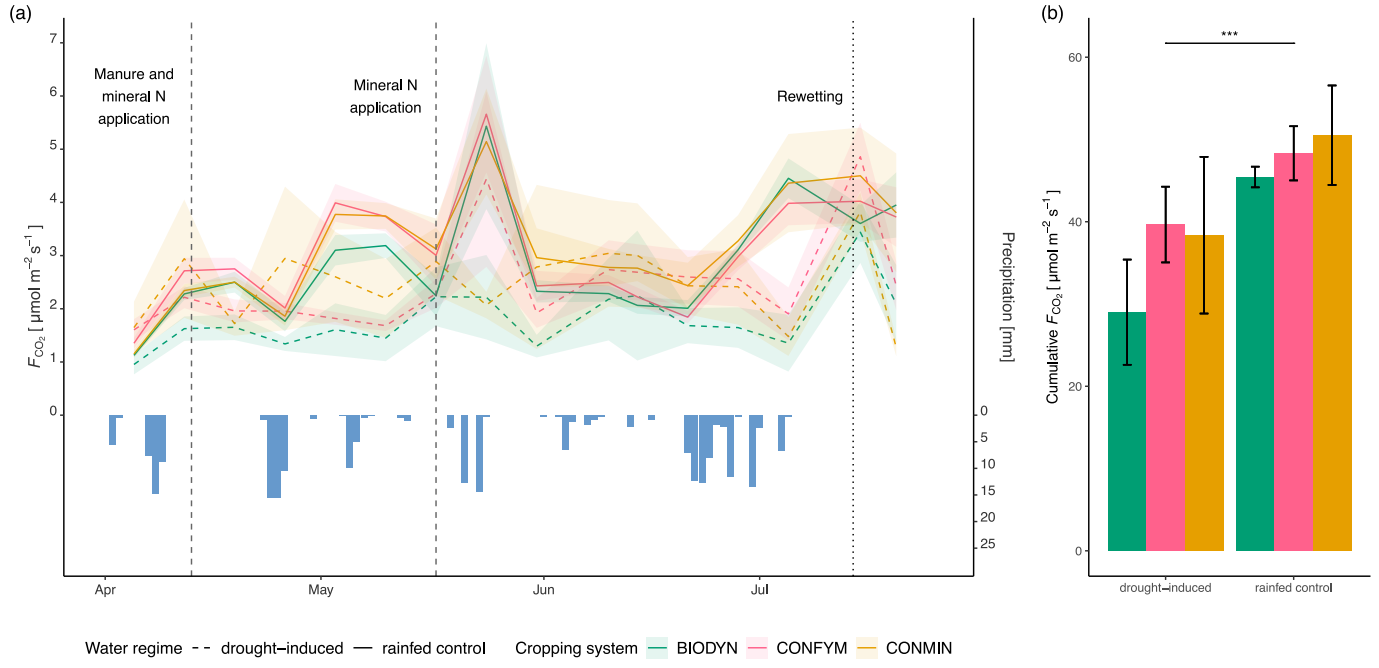

Fig. 3. Soil respiration over time and cumulative in the sheltered and control plots. A) Soil respiration was weekly measured in-situ weekly over the wheat vegetation period up to one week after rewetting and B) cumulatively calculated for the entire drought period. Mean values and standard errors are provided (n=4). Rewetting, manure, and mineral nitrogen (N) applications are indicated by vertical dashed lines. Blue bars represent the total daily precipitation for rainfed control plots. Significant results (ANOVA, $p < 0.001$) are indicated by asterisks (***).

Table 1
PERMANOVA results (F-ratio, p-value, and R^2) showing the effect of drought, cropping system, and sampling date on the prokaryotic and fungal β-diversity during the wheat vegetation period. Differences are based on Bray-Curtis dissimilarities and separately analyzed for the three compartments (i.e., bulk soil, rhizosphere, and root). Heteroscedasticities are indicated as superscript [1]. Values $p < 0.05$ are indicated in bold.

Prokaryotes						
	Bulk soil		Rhizosphere		Root	
	F (p)	R^2	F (p)	R^2	F (p)	R^2
Water regime (W)	1.4 (0.1297)	0.015	3.0 (**0.0069**)	0.031	13.3 (**0.0001**) [1]	0.115
Cropping System (C)	15.1 (**0.001**) [1]	0.307	14.5 (**0.0001**) [1]	0.298	11.5 (**0.0001**)	0.200
Sampling Date (S)	1.4 (0.1545)	0.014	1.2 (0.2151)	0.012	6.4 (**0.0001**)	0.056
W x C	0.9 (0.5698)	0.018	1.1 (0.3161)	0.022	2.0 (**0.0049**)	0.035
W x S	0.7 (0.6629)	0.008	1.0 (0.3359)	0.01	3.7 (**0.0002**) [1]	0.032
C x S	0.8 (0.7643)	0.015	0.8 (0.6262)	0.017	1.3 (0.1062)	0.023
W x C x S	0.7 (0.8695)	0.014	0.7 (0.8467)	0.015	1.1 (0.3557)	0.018
Fungi						
	Bulk soil		Rhizosphere		Root	
	F (p)	R^2	F (p)	R^2	F (p)	R^2
Water regime (W)	5.4 (**0.0001**)	0.057	7.7 (**0.0001**) [1]	0.078	6.2 (**0.0001**) [1]	0.068
Cropping System (C)	10.7 (**0.0001**)	0.225	9.0 (**0.0001**)	0.181	4.5 (**0.0001**)	0.099
Sampling Date (S)	1.8 (**0.0146**)	0.019	4.7 (**0.0001**)	0.047	5.9 (**0.0001**) [1]	0.064
W x C	1.1 (0.3042)	0.023	1.2 (0.1424)	0.024	1.2 (0.0927)	0.027
W x S	1.5 (0.0550)	0.016	2.9 (**0.0001**)	0.029	3.7 (**0.0001**) [1]	0.040
C x S	0.9 (0.7199)	0.019	1.0 (0.3626)	0.021	1.3 (0.0654)	0.028
W x C x S	1.0 (0.4576)	0.021	0.9 (0.7728)	0.017	0.9 (0.6010)	0.020

dissimilarity between the water regimes with proceeding drought was observed mainly for fungi in the rhizosphere and root (Fig. S10).

The CAP using water regime and cropping system as the constraining factors showed distinct clusters between the water regimes during drought in all three cropping systems and in all compartments for fungi and prokaryotes, supported by high and significant reclassification rates (Fig. 4). Thus, in contrast to PERMANOVA, CAP and the associated discriminant analysis could resolve differences between water regimes in all compartments and for both communities. In the bulk soil, the cropping system was the main driver of cluster formation (Fig. 4a and b); in the rhizosphere, the two water regimes already showed more distinct clusters (Fig. 4c and d); in the root, the cluster separation was similar between the two water regimes and the cropping systems (Fig. 4e and f).

A CAP for the bulk soil using the water regime as the constraining factor was conducted to evaluate differences in prokaryotic (Fig. 5a) and fungal β-diversity (Fig. 5b) over time including the period after rewetting. This revealed high reclassification rates for prokaryotes and fungi for both water regimes over the whole experiment with similar differences at each sampling date independent of the cropping system and no apparent recovery after rewetting (Fig. 5). In addition, a CAP constraining by water regime and sampling date (whole drought period versus first and second timepoint after rewetting) performed for each cropping system separately (Fig. S11) revealed distinct clusters for fungal and prokaryotic communities at the first and second timepoint after rewetting in the drought-induced treatment compared to the control for all cropping systems. In the control, samples of the drought period and one week after rewetting could hardly be differentiated which was not apparent for the samples from induced drought. PERMANOVA, run for the two sampling dates after rewetting, revealed strong differences in fungal β-diversity and comparatively minor differences in prokaryotic β-diversity between drought-induced and control plots after rewetting. No interactions were reported between the cropping system and water regime after the rewetting (Table S6).

3.7. Drought affects certain taxa differently in different cropping systems

Around 3 % (23 out of 696), 13 % (91), and 23 % (161) of the prokaryotic genera, and 6 % (28 out of 439), 14 % (61), and 11 % (49) of the fungal genera were significantly ($q < 0.05$) altered by drought across all cropping systems in the bulk soil, rhizosphere, and roots, respectively (Fig. S12). Genera sensitive to drought were spread over the taxonomic tree, but drought stress tended to increase the relative abundance of genera assigned to *Actinobacteriota* and decrease genera assigned to *Bacteroidota* and *Planctomycetota* in all compartments. In bulk soil, *Cyanobacteria* decreased and *Glomeromycota* increased (Fig. S12).

Including all compartments, 8 % (54 out of 696) of the prokaryotic genera and 5 % (20 out of 439) of the fungal genera showed a significant ($q < 0.1$) cropping system-dependent response to drought (Fig. 6). Genera with a cropping system-dependent response to drought in the bulk soil included but were not limited to *Rhizophagus*, *Microdominikia* (both *Glomeromycota*), *Methanobrevibactera* (*Euryarchaeota*), *Trichococcus, Christensenellaceae R-7, Saccharofermentans, Fastidiosipila, Ercella* (all *Firmicutes*), *Levilinea, Leptolinea* (both *Chloroflexi*), *Roseimarnus, Proteinphilum, Fermentimonas* (all *Bacteroidota*), and *Glycomyces* (*Actinobacteriota*). In the rhizosphere, differentially responsive genera included *Gremmenia, Blumeria* (both *Ascomycota*), *Variovorax, Massilia* (both *Proteobacteria*), *Proteiniphilum* (*Bacteroidota*), *Actinomadura* and *Lechevalieria* (both *Actinobacteriota*). In the roots, differentially responsive genera included for example *Blumeria* (*Asocomycota*), *Paracoccus* (both *Proteobacteria*), *C. Desulforudis, Sedimentibacter*, *Ruminiclostridium* (all *Firmicutes*), *Solitalea, Proteiniphilum* (both *Bacteriodetes*), *Streptomyces, Kitosatospore, Umezawaea,* and *Salinispora* (all *Actinobacteria*). Results on other taxonomic levels can be found in Supplementary Data 1.

Cropping systems had a significant influence on the prokaryotic copiotrophs:oligotrophs ratio in the bulk soil and rhizosphere (Fig. S13). A significantly higher copiotrophs:oligotrophs ratio was found for drought when compared to the rainfed control in the bulk soil and rhizosphere at the third sampling date. After the rewetting, a higher copiotrophs:oligotrophs ratio was detected (i.e., only measured in bulk soil). A significantly increased ratio of copiotrophs:oligotrophs was found in the roots under drought compared to the control at the second and third sampling date (Fig. S13).

4. Discussion

4.1. Implementation of drought

Drought conditions were successfully induced at field scale (Fig. 2, Fig. S2), with a reduction in water availability characteristic of

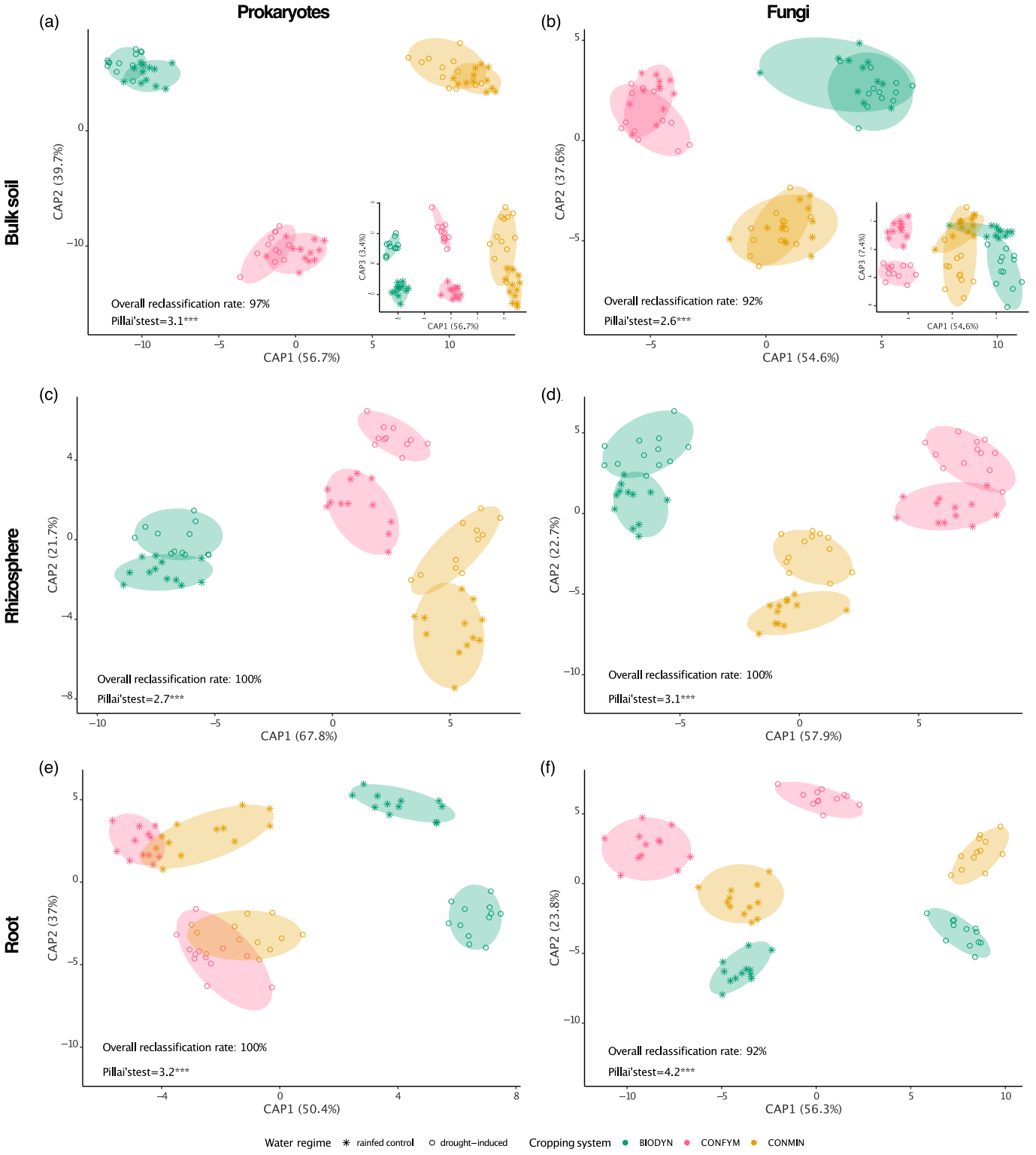

Fig. 4. Effects of drought and cropping system on prokaryotic and fungal β-diversity during the drought period. Differences are displayed as canonical analysis of principal coordinates (CAP) maximizing discrimination between water regimes and cropping systems. The CAP overall reclassification rate in percentage, Pillai's trace statistics, and statistical significance ($p < 0.001$ ***) are provided in each plot. Panels represent differences in prokaryotic communities in bulk soil (A), rhizosphere (C), and roots (E) as well as fungal communities in bulk soil (B), rhizosphere (D), and roots (F). The amount of between-group variation of each CAP axis is provided in parentheses. For bulk soil, the third dimension is provided to show the separation by the drought treatment.

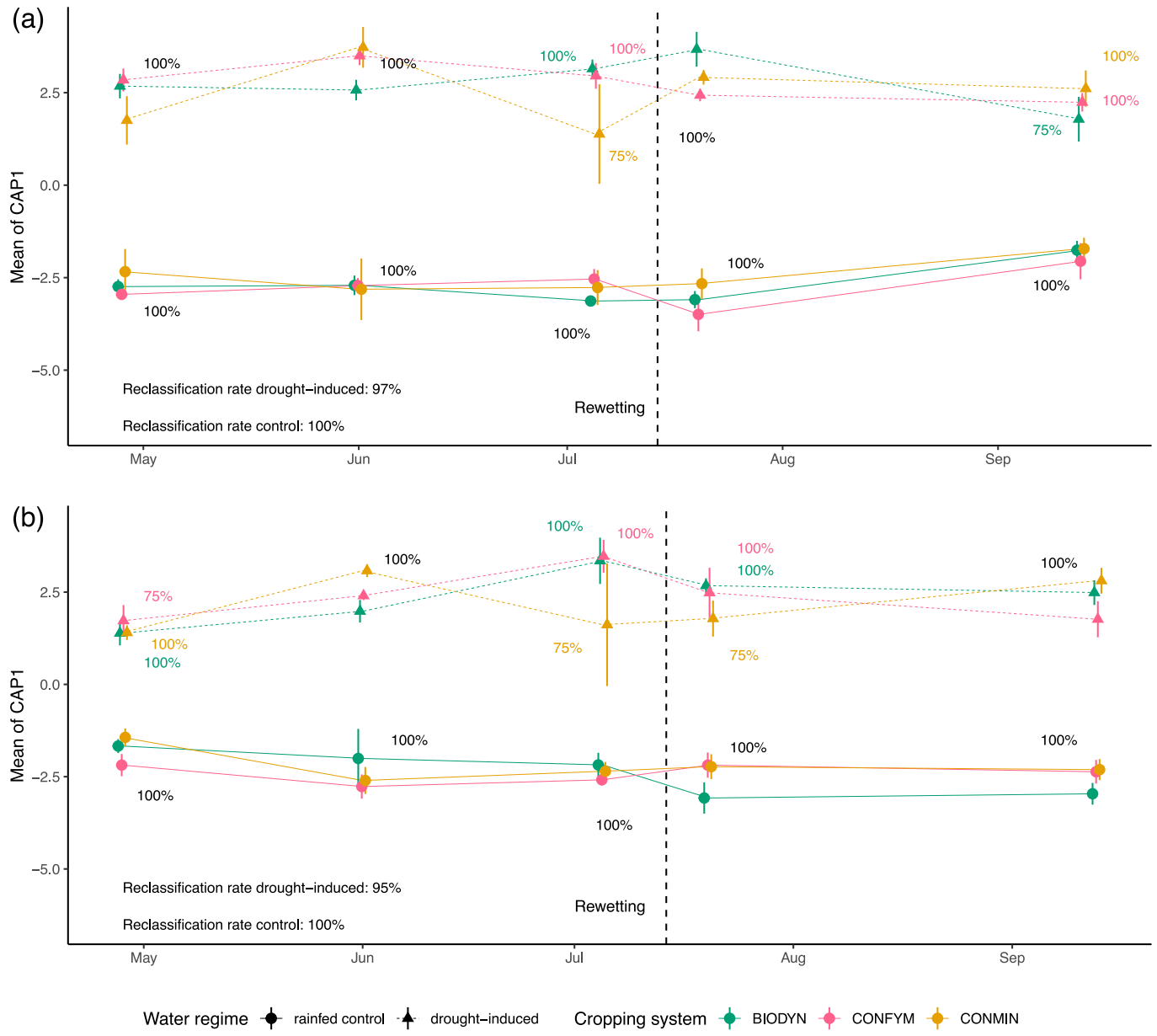

Fig. 5. Effects of drought on prokaryotic and fungal β-diversity during drought and after rewetting. Differences are displayed as means and standard errors of the first canonical axis from the canonical analysis of principal coordinates (CAP) maximizing discrimination between water regimes (n = 4). The CAP overall reclassification rate in percentage, Pillai's trace statistics, and statistical significance ($p < 0.001$ ***) are provided in each plot. Reclassification rates for each water regime to their water regime at each sampling timepoint and cropping system are provided and displayed in case of differences between cropping systems in the respective color. Panels represent differences in prokaryotic communities (A) and fungal communities (B) in bulk soil. The amount of between-group variation of each CAP axis is provided in parentheses.

comparatively severe drought stress [38,45]. In contrast to our expectations, there was no significant effect of the different cropping systems with distinct SOC contents on decreasing GWC (Fig. 2). Although the magnitude of the water content decrease differed between the measurement methods (Fig. 2, Fig. S2), they all showed a continuous decrease in water content in the sheltered plots. A recent short-term, partial sheltering study in two cropping systems of the same field found different GWC reductions between the cropping systems under moderate drought but not under severe drought [38]. Compared with the former study, the drought implemented in the current study was longer, more severe and differences between sheltered and control plots were more pronounced. Studies showed that the effect of SOC content on water retention decreased with decreasing soil water potential [68,69], resulting in little impact on water retention under severe drought. In addition, SOC contents have limited effects on soil water retention in soil rich in silt and clay minerals [68,69]. Since the soil at the DOK trial is a Haplic Luvisol and contains around 72 % silt and 16 % clay [29], the potential of SOC content to increase the soil water retention in this field experiment is likely limited. It is important to note that the soil C content in this field experiment is low compared to other agricultural field sites [70], which might further influence the effect of SOC on soil water retention.

Additional to the reduction of the soil water content, the sheltering also increased the soil and air temperature (Figs. S3 and 4). Although the air temperature was not strongly affected by the sheltering, this can be an undesirable side effect of rainout shelters. However, one could also argue that droughts not only occur due to reduced precipitation patterns but also due to increased evapotranspiration driven by rising

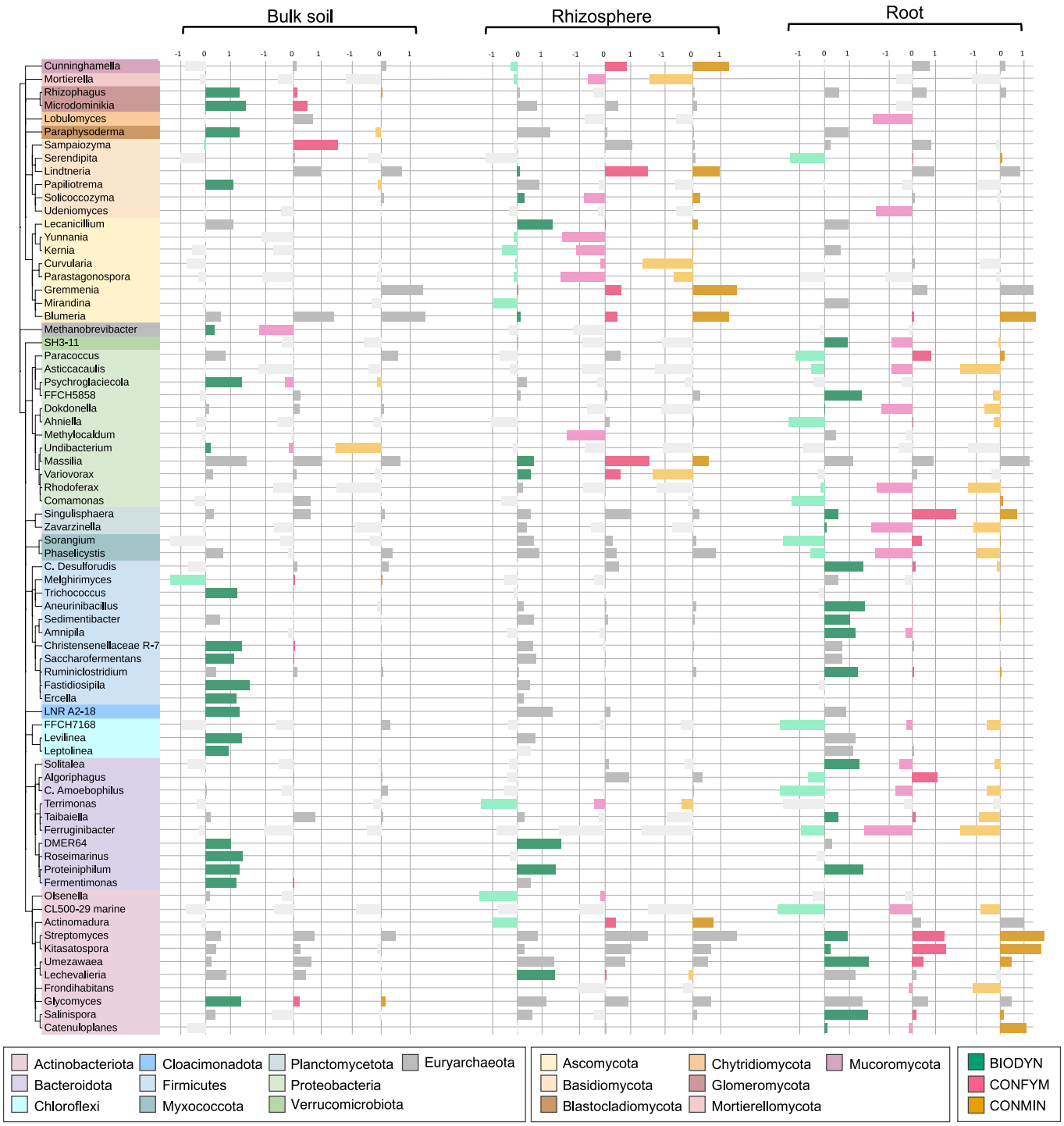

Fig. 6. Taxonomic tree displaying prokaryotic and fungal genera in bulk soil, rhizosphere, and roots showing a significant interaction between drought response and cropping system. Genera showing a significant (PERMANOVA, $q < 0.1$) interaction are color-coded by the corresponding cropping system, and grey bars are non-significant interactions. Bar plots show the z-transformed relative change in abundance between drought-induced and rainfed treatment of genera enriched or depleted under drought in the respective cropping systems. Color ranges identify corresponding phyla.

temperatures [46,71]. Since the beginning of the field experiment in 1978 the mean annual temperature has risen from 9.7 °C to 10.9 °C [29], and this trend is expected to persist [1]. Therefore, the rainout shelters might well represent the changing conditions caused by climate change. Another side effect of the warmer temperature below the shelters is accelerated plant development, which could influence the differences in microbial communities below and outside the shelters. One option would be to conduct the samplings according to the plant stage, which however could cause additional artificial effects (i.e., precipitation, temperature etc.). Since the plant stages were not very different between the control and drought-induced plots, we can assume that the effects of different plant stages between the water regime plots on soil microbial communities were probably not very pronounced. It is crucial to note that the effects of time, phenology, and season on plants but also soil properties including microbes are difficult to disentangle. Sampling was conducted three times across the wheat vegetation period since plants

might adapt microbial recruitment to their plant-stage specific requirements [72] and temporal effects on soil microbes have frequently been reported [73,74].

4.2. Drought affects aboveground biomass and nutrients

The increased air temperature of 0.8 ± 0.03 °C below the rainout shelters during winter led to enhanced plant height and biomass at stem elongation. However, drought reduced plant height at flowering and ripening as reported in the literature [75], while dry biomass was not affected and thus contradicted the results of Wittwer et al. (2023) [76]. Khadka et al. (2020) [75] argued that for example, drought-tolerant varieties tend to grow smaller and increase their root biomass to access deeper soil layers. This potentially helped the plants to maintain aboveground biomass under drought. At the last sampling date, sheltered wheat plants were at Zadok stage 93 potentially resulting in the loss of part of grains before sampling. Nevertheless, there was no significant increase in volunteer grain recorded in fall 2022 in the previously sheltered area (data not shown). It is crucial to mention that plant biomass was measured on a small area (three wheat rows of 17.5 cm × 8 cm), which might not accurately represent crop yields of the entire field. Plant height differences between the conventional and biodynamic systems were caused by the application of plant growth regulators in conventional systems. Yet, plants in BIODYN did not differ in plant height between the water regimes. The grown variety Wiwa was specifically bred for organic management, which could result in an improved adaptation to organic systems and subsequently better stress tolerance [77]. The impact of drought on plants might depend further on the timing, duration, and severity of the drought [78].

Reductions in plant nutrient contents (e.g., nitrogen, potassium, magnesium; Fig. S5), which are normally highly mobile under wet conditions, are commonly observed under drought due to limited mobility under dry conditions [79,80]. Additionally, the decomposition of organic nutrients is often reduced under drought due to reduced soil microbial activity (Fig. 3) [79]. However, shoot magnesium at ripening was significantly increased under drought compared to the control (Fig. S5), which might indicate a different allocation of magnesium to grains during wheat ripening under drought conditions. Magnesium is considered as important for water-use-efficiency and plays a crucial role in photosynthesis [81]. Studies on the interaction of magnesium and drought are however still scarce [79]. The reduction of the shoot nitrogen content at ripening was only significant in CONFYM and not in the other systems. Since stacked manure and the mineral nitrogen fertilizer were applied in October 2021 and during the wheat vegetation period (Table S1), respectively, the plant mineral nitrogen nutrition was possibly higher in CONFYM compared to the other systems. The diffusion and absorption of phosphorus also depend on the soil water content (Fig. S5), which then leads to a phosphorus deficit in the plant and accumulation in soils (Fig. S5, Table S3) [79]. No effect of the phosphorus deficit under drought was found in the ears at flowering in BIODYN and CONMIN, and in CONFYM the control showed the highest ear phosphorus contents. This might be caused by the high available phosphorus contents in soil found in CONFYM soils compared to the other systems (Table S3), and these might be less available under drought [79]. The higher shoot carbon contents at stem elongation below the shelters were possibly caused by higher temperatures during winter as mentioned above improving the wheat growth.

4.3. Drought alters microbial composition and soil activity, but not abundance

Drought altered soil fungal and prokaryotic community structures in all studied compartments although the effect observed in the bulk soil compartment was not very strong (Table 1, Fig. 4). Drought effects on microbial communities are in accordance with previous studies reporting on the effects of drought on soil microbes [3,4]. CAP ordinations showed distinct microbial communities between the drought-induced and control plots in all cropping systems (Fig. 4), which was largely confirmed by the PERMANOVA results except for prokaryotes in the bulk soil (Table 1); for the latter, effects of drought might have been masked by other more dominant drivers such as cropping system and soil texture.

In contrast to microbial community structure, prokaryotic and fungal abundance measured by ribosomal gene copy numbers was not affected by drought (Fig. S7). However, when taking into account the estimated copiotroph:oligotroph ratio for prokaryotes, which was significantly increased under drought at the last measured drought timepoint (Fig. S13), the cell abundance of prokaryotes might have decreased upon drought. Other studies show contrasting results on microbial abundance or biomass [10,11,82,83], ranging from a decrease, to no effects, or even an increase under drought. The conflicting findings may depend on the evaluation method, soil type, drought severity, and duration. However, it is important to note that relic DNA might accumulate under drought because of the reduced microbial activity [84], which could disguise drought effects on microbial abundance. Drought reduced soil respiration in all cropping systems (Fig. S7).

Higher copiotrophic/oligotrophic ratios under drought are contradictory to the hypothesis of Naylor and Coleman-Derr (2018) [15] and previous results in forests and grasslands showing that oligotrophs thrive under drought conditions [15,85,86]. Opposed to forest and extensively used grassland soils, agricultural cropping systems are frequently fertilized, which might influence how oligotrophs and copiotrophs respond to drought.

A reduction of soil activity under reduced water availability was observed for all cropping systems (Fig. 3), which is in line with other studies [10,11]. Interestingly, soil respiration was lowest in the BIODYN treatment, although other studies reported higher respiration rates in organically managed cropping systems [27,28,38]. However, these studies measured basal respiration under controlled conditions instead of *in-situ* soil respiration in field soils with plant growing as done in this study. Although an equilibration period is often conducted before measuring basal respiration, the disturbance of the soil through sieving might lead to higher soil organic matter decomposition [87], and soil organic carbon has been reported to be higher in the biodynamic system than in other systems of the DOK trial [29]. The interactive effect between drought and cropping system, which was however not supported by the subsequent post hoc tests, might indicate a higher reduction of soil respiration under drought in the biodynamic system compared to the conventional systems (Fig. 3b). Contrasting to this finding, higher root carbon is commonly observed in organic systems [88,89], which would probably increase soil respiration. Potentially there was a stronger reduction of root biomass or microbial activity under drought in the biodynamic system. Nevertheless, all cropping systems showed a reduction of microbial activity under drought (Fig. 3).

4.4. Drought in cropping systems affects soil fungi more strongly than prokaryotes

In contrast to our first hypothesis, drought affected soil fungi more strongly than prokaryotes in the bulk soil and rhizosphere but not the roots (Table 1, Fig. 5). Previous studies observed stronger drought effects on prokaryotic community composition [3,4]. Yet, many of these studies were performed either in greenhouse pots or in grasslands, which are managed differently than arable cropping systems. Fungal hyphal networks are crucial for plant water acquisition [12], and these networks might be more disturbed in cropping systems compared to grasslands by management practices such as soil tillage and mechanical weeding [90]. We did not find a drought effect on fungal abundance, as assessed by rRNA gene copy numbers (Fig. S7). However, it was shown that hyphal networks do not necessarily contain nucleoid acids and rRNA gene copy numbers might therefore not correlate well with hyphal length [91]. The F/P ratio was lowest in BIODYN in the bulk soil

(Fig. S7). Since mechanical weeding is performed twice in BIODYN in addition to tillage, although only to 2 cm, the fungal networks might have been disrupted more strongly in this system. A study by Frey et al. (1999) [91] showed that the fungal hyphal length was more strongly affected by limited soil moisture under conventional tillage compared to no-till systems.

Our findings are nevertheless in accordance with a recent spring wheat field experiment, which showed a stronger drought influence on soil fungi compared to prokaryotes [92], arguing that fungi are more sensitive to changes in plant exudation, particularly carbon. Two other field studies in cropping systems with wheat, sugar beet, and maize found a stronger drought response of bacterial communities compared to fungal [3,93], implying that response to drought also depends on other variables such as crop, soil properties, climate, drought severity, and other agricultural practices. Multi-trophic interactions might also influence the microbial drought response such as reduction or shifts of protists or nematodes, which have been shown to be drought-sensitive [94,95]. Such effects might subsequently affect feeding pressure or release nutrients to soils.

In this context, it is important to mention that a stronger shift of microbial communities in response to drought could also suggest a higher adaptation potential rather than a lack of resistance to drought. Another potential explanation for the weaker drought response of prokaryotes compared to fungi could be attributed to preceding summer droughts in 2018 and 2019, which might have led to an adaptation of bacteria to drought, as the fast adaptation of bacteria towards stress is well-known [96]. Prokaryotes might be protected from drought within microaggregates [14], resile in small pores, or become dormant [9,13].

Overall, this field experiment showed that soil fungi might be more affected by drought in arable cropping systems compared to prokaryotes in the bulk soil and rhizosphere possibly due to soil disturbance. It is important to note that microbial drought response further depends on other factors like soil type, texture, aggregation, climate, drought severity, and multi-trophic interactions [4,36].

4.5. Drought effects on prokaryotes and fungi increase with increasing proximity to plants

There was a stronger influence of drought on microbial communities more closely associated with plant roots (Table 1, Fig. 4), revealing more taxa sensitive to drought in the rhizosphere and root when compared to the bulk soil (Fig. S12, Supplementary Data 1). This finding is in accordance with our second hypothesis (ii) and previous studies [3,21,36]. This effect was stronger for prokaryotes than for fungi. The stronger response of root-associated microbes is likely caused by a combination of direct effects of water scarcity on the microbes and indirect effects mediated through the drought-affected plants [97]. On the one hand, drought-stressed plants can alter rooting depth and density [98], consequently changing the microbial habitat. On the other hand, metabolic changes in drought-stressed plants can alter rhizodeposition and thereby affect soil microbial communities, especially in proximity of roots [23]. Through this process, plants can select for root microbes that increase plant drought tolerance [23,97]. Increased soil water contents have been found in the rhizosphere compared to bulk soil possibly because of mucilage exudated by roots [99], which might result in a lower adaption of microbes to drought in the rhizosphere compared to the bulk soil, where fluctuation in water availability might be more frequent. There might be water competition between microbes and plants in the rhizosphere, thus rhizosphere microbes might experience more severe water limitations. Moreover, plants accumulate osmolytes in roots to sustain root growth under low soil water potential [100], which might additionally influence root endophytes. However, specific interactions and plant-microbial pathways under drought are still largely unknown, especially under field conditions.

4.6. Cropping-system dependent resistance to drought

Overall, the effects of drought on community structure were largely independent of the cropping systems, except for the root prokaryotes (Table 1), not providing strong support for the third hypothesis (iii). Resistance is defined in this study as the lack of shift of the microbial composition to drought. These results contradict previous results of a pot experiment, which found significant cropping system effects on the drought response of bacterial composition using soils from a conventionally and organically managed field trial [36]. The interactive effect was stronger in sandy soils compared to loamy soils [36] but no organic fertilizers were applied in the organic cropping system. However, results from pot experiments often do not translate well to conditions in the field, potentially explaining some of these discrepancies. Other pot experiments found a few interactions between drought and the addition of organic amendments on enzyme activities and microbial composition through phospholipid fatty acids [33–35], mentioning a slower drying in amended soils but when reaching the dry state they exhibited similar behaviors. Other field studies found no effect of organic management or reduced tillage on the reduction of decomposition activity under drought [45,101]. Furthermore, a partial, short-term sheltering experiment in the same long-term trial found no strong interactive effect of cropping system and experimental drought under moderate drought [102], supporting our findings.

Although the cropping-system dependent effects of drought on the microbial community were relatively small, several genera showed a system-specific response (Fig. 6). *Streptomyces* and *Kitasatospora* were enriched in CONFYM and especially CONMIN under drought compared to BIODYN. Both are potential plant growth promoting (PGP) bacterial genera known to produce the phytohormone auxin, siderophores, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase [103]. Auxin can increase the growth of lateral roots and root hairs [104]. Plant ethylene contents, which can decrease plant and root growth under stress, are reduced by the ACC deaminase and thereby increase tolerance to stress [105]. Siderophores produced by PGP bacteria can solubilize and sequester iron in soils helping plants with the iron uptake and can be involved in the suppression of plant pathogens [106]. *Streptomyces,* often enriched under drought (Fig. S12), are considered to be important for plant drought tolerance and are successful in colonizing root tissue under stress [107]. *Actinomadura* known for siderophore and auxin production was additionally enriched in CONFYM and CONMIN compared to BIODYN [103]. Other potential PGP bacteria particularly enriched under drought in CONFYM were *Massilia* and *Paracoccus* [103,108]. *Variovorax*, which was enriched in CONFYM and BIODYN, has been described to improve plant drought tolerance exhibiting similar mechanisms as mentioned above [109]. In the BIODYN treatment, the genera *Aneurinibacillus, Glycomyces, Lechevalieria, Salinispora,* and *Umezawaea* were enriched under drought, which contain species potentially promoting plant growth and are often found in compost [103,110]. Some species in these genera are known for auxin and siderophore production, and ACC deaminase activity [111] but also feature biocontrol activity [110–112]. For soil fungi, the genera *Blumeria*, and *Gremmenia* were increased particularly in CONMIN under drought compared to the other cropping systems (Fig. 6). Both are potential plant pathogens, and *Blumeria graminis* is known to infest wheat [113,114], indicating that plants in CONMIN under drought might have experienced a higher pathogen pressure. However, this higher pathogen pressure in CONMIN under drought was not visible on aboveground plant leaves at all samplings (data not shown). *Lecanicillium*, *Papiliotrema*, *Microdominikia*, and *Rhizophagus* were enriched under drought in BIODYN. These genera are known to contain PGP species [115–119]. Rhizophagus, for example, are arbuscular mycorrhizal fungi known to be able to improve plant drought tolerance [115].

Interestingly, several genera that increased under drought in BIODYN compared to the other cropping systems are known to contain facultatively or obligate anaerobic species (i.e., *Fermentimonas,*

Proteiniphilum, Roseimarinus, Solitalea, Leptolinea, Levilinea, Ercella, Fastidiosipila, Ruminiclostridium, Saccharofermentans, Christensenellaceae R-7 group, Sedimentibacter, Candidatus Desulforudis, Trichococcus, Methanobrevibacter; Fig. 6) [120,121]. Many of these genera have been found in slurry or animal rumen and are involved in fermentation and methanogenesis [122,123]. Indeed, slurry was applied in February and March in the BIODYN treatment but not in CONFYM and CONMIN. However, this relative increase of species involved in methanogenesis in BIODYN soils under drought did not increase *in-situ* methane emissions (data not shown), which suggests that the increased relative abundance did not translate into increased activity, either because these genera were inactive or dead [45,84].

In this study, we defined resistance as the ability to tolerate drought by not changing community composition [25]. Hence, a more pronounced shift in microbial community structure upon drought would suggest lower resistance to drought, while no or a small shift would indicate stronger resistance. Nevertheless, each cropping system maintained a unique microbial community also under drought stress (Fig. 4), which could hold different capacities to cope with water limitations. Therefore, it remains to be elucidated whether increases or decreases of specific taxa in one versus the other cropping system implies lower resistances in one system than the other, or if it actually represents some adaptation mechanisms that can improve drought tolerance of the system.

In summary, all cropping systems showed under drought enrichments of some PGP genera potentially involved in the improvement of plant drought tolerance, especially of the phylum *Actinobacteriota*. Generally, fungal genera possibly involved in improving plant drought tolerance were enriched in BIODYN. Moreover, microbial communities were similarly affected by drought in all cropping systems. Hence, we found no clear indication that the application of composted or stacked manure in BIODYN and CONFYM, the associated increase in SOC [29] and microbial diversity [24], the reduction of pesticide application, or other factors like the biodynamic preparations in BIODYN could increase microbial resistance to drought. Additionally, this long-term field trial already includes some regenerative practices such as shallow tillage, cover cropping, and incorporation of grass-clover into the crop rotation in all cropping systems. Those practices might have already improved microbial resistance to drought and still, shifts of microbial communities were recorded. However, we did not find a strong indication of different resistances of the microbial communities, and GWC reduction did not differ under drought between the manure-treated and minerally fertilized systems. Yet, cropping systems still harbour distinct prokaryotes and fungi under severe drought and these distinct communities might feature contrasting potentials to cope with drought. It is important to note that this study is confined to one climate, crop, and soil type.

4.7. Cropping system-dependent resilience to drought

Despite the effect of drought on the bulk soil prokaryotes and fungi was not very strong, a drought legacy effect one week and about two months after rewetting of the bulk soil was clearly detectable (Fig. 5, Fig. S11, Table S6), which is supported by previous studies [5,107]. However, prokaryotic and fungal communities did not show distinct resilience patterns depending on the cropping system, with resilience being defined here as the return of the microbial community composition to the rainfed control conditions. Therefore, we have to reject our fourth hypothesis that different cropping systems might show different capacities for resilience (iv). Some pot studies found comparable resilience in soils with and without organic amendments assessed by enzyme activities, basal respiration, and phospholipid acids [33,35], while another study found differences in resilience patterns using molecular analysis [37]. Our findings that no distinct microbial resilience patterns were observed align with the GWC results. The increase in soil water content after rewetting did not differ between the cropping systems (Fig. 2).

There is a limited number of studies that have assessed microbial resilience to drought in contrasting cropping systems, particularly involving plants and at field-scale. To the best of our knowledge, this study is the first field-scale experiment to assess soil microbial resilience, assessed as structural composition, after severe drought comparing organic and conventional long-term cropping systems. This study indicates that the application of organic amendments in the form of farmyard manure in organic and mixed conventional cropping systems, or the reduction of pesticide application or factors like biodynamic preparations might have limited effects on microbial resilience after drought. This is supported by the finding that we did not find increased soil moisture in one over the other cropping systems after rewetting (Fig. 1). However, the effect may depend on climatic conditions, soil type, and crop.

5. Conclusions

First, our results suggest that in arable cropping systems soil fungi might be less resistant to drought compared to prokaryotes possibly because of frequent soil disturbances or stronger interaction with plant exudates. Secondly, this study indicates that cropping systems considered to promote soil biodiversity and SOC content, such as organic cropping systems, might not be able to mitigate the impact of severe drought on soil microbial composition. However, these results were obtained from a Halpic Luvisol with high silt contents in a temperate region under wheat cultivation, and may not apply in, for example, sandy soils in other climate regions or cropping systems. Since this study focused on assessing the effects of drought on taxonomic diversity, our conclusions about microbiome-mediated changes in soil functions under drought are still limited. Approaches that can link shifts in diversity to shift in the underlying functional potential – such as metagenomics – could shed more light on the potential consequences of the compositional changes. Given that this field trial already includes some regenerative practices in all cropping systems, comparison to other cropping systems including more conventional practices such as conventional tillage, fallows, or monocropping would put the cropping systems in the DOK trial into a broader perspective. Finally, stronger drought effects were found for microbes more closely associated with roots, which emphasizes the importance of plant-microbe interactions. Additional studies are needed to examine rhizodeposition patterns under drought in different cropping systems in order to better understand the relevance of these interactions to mitigate the impact of climate change stressors.

CRediT authorship contribution statement

Elena Kost: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Dominika Kundel:** Writing – review & editing, Methodology, Investigation. **Rafaela Feola Conz:** Writing – review & editing, Investigation. **Paul Mäder:** Writing – review & editing, Methodology. **Hans-Martin Krause:** Writing – review & editing, Methodology. **Johan Six:** Writing – review & editing, Supervision, Project administration. **Jochen Mayer:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition. **Martin Hartmann:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

Competing interests

The authors declare no competing interests.

Data availability

Raw sequence data were deposited in the European Nucleotide Archive under the accession number PRJEB73799.

Funding

This research was funded through the 2019–2020 BiodivERsA joint call for research proposals under the BiodivClim ERA-Net COFUND program, with contributions from the funding organizations Swiss National Science Foundation SNSF (31BD30_193666), Agencia Estatal de Investigacion AEI (SPCI202000X120679IV0), Agence nationale de la recherche ANR (ANR-20-EBI5-0006), Federal Ministry of Education and Research BMBF (16LC2023A), and General Secretariat for Research and Innovation GSRI (T12EPA5-00075).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

We thank members of the research groups at FiBL Frick (Group Soil fertility & Climate), Agroscope Zürich (Group Water Protection and Substance Flows), Uni Kassel (Group soil biology and plant nutrient), and ETH Zürich (Sustainable Agroecosystems Group) for their contributions to this study. We are especially grateful to Hans-Ulrich Zbinden, Frédéric Perrochet, Moritz Sauter, Adrian Lustenberger, Matti Barthel, Charles Nwokoro, Tim Juchli, Noah Schweizer, Moritz Bach, and Bernhard Stehle for their support during fieldwork. We are grateful to all the helpers during sampling and *in-situ* data collection including Matthias Lang, Sabrina Niehaus, Juliana Jäggle, Sarah Symanczik, Marijn Van de Broek, Lian Tengxiang, Tania Galindo, Tabata Bublitz, and Astrid Jäger. We are also grateful to Rafaela Feola Conz, Matti Barthel, Britta Jahn-Humphrey, and the soil and elemental analysis group at Agroscope for their technical and work support in the laboratory. Finally, we would like to acknowledge Maria Domenica Moccia at the Functional Genomics Center Zurich (FGCZ) for the amplicon sequencing service on the Illumina MiSeq v3 platform.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2024.103690.

References

[1] IPCC, Summary for Policymakers, in: H. Lee, J. Romero (Eds.), Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, vol. 335, IPCC, Genev, 2023, pp. 1–34.

[2] C. Lesk, P. Rowhani, N. Ramankutty, Influence of extreme weather disasters on global crop production, Nature 529 (2016) 84–87, https://doi.org/10.1038/nature16467.

[3] D. Naylor, S. Degraaf, E. Purdom, D. Coleman-Derr, Drought and host selection influence bacterial community dynamics in the grass root microbiome, ISME J. 11 (2017) 2691–2704, https://doi.org/10.1038/ismej.2017.118.

[4] R. Ochoa-Hueso, S.L. Collins, M. Delgado-Baquerizo, K. Hamonts, W.T. Pockman, R.L. Sinsabaugh, M.D. Smith, A.K. Knapp, S.A. Power, Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents, Global Change Biol. 24 (2018) 2818–2827, https://doi.org/10.1111/gcb.14113.

[5] C. Preece, E. Verbruggen, L. Liu, J.T. Weedon, J. Peñuelas, Effects of past and current drought on the composition and diversity of soil microbial communities, Soil Biol. Biochem. 131 (2019) 28–39, https://doi.org/10.1016/j.soilbio.2018.12.022.

[6] J.P. Schimel, Life in dry soils: effects of drought on soil microbial communities and processes, Annu. Rev. Ecol. Evol. Syst. 49 (2018) 409–432, https://doi.org/10.1146/annurev-ecolsys-110617-062614.

[7] V. Sandhya, S.Z. Ali, The production of exopolysaccharide by Pseudomonas putida GAP-P45 under various abiotic stress conditions and its role in soil aggregation, Microbiology (N. Y.) 84 (2015) 512–519, https://doi.org/10.1134/S0026261715040153.

[8] M. Potts, Desiccation tolerance of prokaryotes, Microbiol. Rev. 58 (1994) 755–805, https://doi.org/10.1128/mmbr.58.4.755-805.1994.

[9] S.E. Jones, J.T. Lennon, Dormancy contributes to the maintenance of microbial diversity, Proc. Natl. Acad. Sci. USA 107 (2010) 5881–5886, https://doi.org/10.1073/pnas.0912765107.

[10] H. Azarbad, P. Constant, C. Giard-Laliberté, L.D. Bainard, E. Yergeau, Water stress history and wheat genotype modulate rhizosphere microbial response to drought, Soil Biol. Biochem. 126 (2018) 228–236, https://doi.org/10.1016/j.soilbio.2018.08.017.

[11] C. Ren, J. Chen, X. Lu, R. Doughty, F. Zhao, Z. Zhong, X. Han, G. Yang, Y. Feng, G. Ren, Responses of soil total microbial biomass and community compositions to rainfall reductions, Soil Biol. Biochem. 116 (2018) 4–10, https://doi.org/10.1016/j.soilbio.2017.09.028.

[12] M.F. Allen, Mycorrhizal Fungi: highways for water and nutrients in arid soils, Vadose Zone J. 6 (2007) 291–297, https://doi.org/10.2136/vzj2006.0068.

[13] J. Schimel, T.C. Balser, M. Wallenstein, Microbial stress-response physiology and its implications for ecosystem function, Ecology 88 (2007) 1386–1394, https://doi.org/10.1890/06-0219.

[14] E.I. Pujol Pereira, H. Chung, K. Scow, J. Six, Microbial communities and soil structure are affected by reduced precipitation, but not by elevated carbon dioxide, Soil Sci. Soc. Am. J. 77 (2013) 482–488, https://doi.org/10.2136/sssaj2012.0218.

[15] D. Naylor, D. Coleman-Derr, Drought stress and root-associated bacterial communities, Front. Plant Sci. 8 (2018) 1–16, https://doi.org/10.3389/fpls.2017.02223.

[16] M. Hartmann, J. Six, Soil structure and microbiome functions in agroecosystems, Nat. Rev. Earth Environ. 4 (2023) 4–18, https://doi.org/10.1038/s43017-022-00366-w.

[17] N. Ortiz, E. Armada, E. Duque, A. Roldán, R. Azcón, Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains, J. Plant Physiol. 174 (2015) 87–96, https://doi.org/10.1016/j.jplph.2014.08.019.

[18] E. Ngumbi, J. Kloepper, Bacterial-mediated drought tolerance: current and future prospects, Appl. Soil Ecol. 105 (2016) 109–125, https://doi.org/10.1016/j.apsoil.2016.04.009.

[19] R. Lata, S. Chowdhury, S.K. Gond, J.F. White, Induction of abiotic stress tolerance in plants by endophytic microbes, Lett. Appl. Microbiol. 66 (2018) 268–276, https://doi.org/10.1111/lam.12855.

[20] J. Edwards, C. Johnson, C. Santos-Medellín, E. Lurie, N.K. Podishetty, S. Bhatnagar, J.A. Eisen, V. Sundaresan, L.D. Jeffery, Structure, variation, and assembly of the root-associated microbiomes of rice, Proc. Natl. Acad. Sci. USA 112 (2015) 911–920, https://doi.org/10.1073/pnas.1414592112.

[21] C. Santos-Medellín, J. Edwards, Z. Liechty, B. Nguyen, V. Sundaresan, Drought stress results in a compartment-specific restructuring of the rice root-associated microbiomes, mBio 8 (2017) 1–15, https://doi.org/10.1128/mbio.00764-17.

[22] D.L. Jones, C. Nguyen, R.D. Finlay, Carbon flow in the rhizosphere: carbon trading at the soil-root interface, Plant Soil 321 (2009) 5–33, https://doi.org/10.1007/s11104-009-9925-0.

[23] A. Williams, F.T. de Vries, Plant root exudation under drought: implications for ecosystem functioning, New Phytol. 225 (2020) 1899–1905, https://doi.org/10.1111/nph.16223.

[24] M. Hartmann, B. Frey, J. Mayer, P. Mäder, F. Widmer, Distinct soil microbial diversity under long-term organic and conventional farming, ISME J. 9 (2015) 1177–1194, https://doi.org/10.1038/ismej.2014.210.

[25] B.S. Griffiths, L. Philippot, Insights into the resistance and resilience of the soil microbial community, FEMS Microbiol. Rev. 37 (2013) 112–129, https://doi.org/10.1111/j.1574-6976.2012.00343.x.

[26] A. Shade, H. Peter, S.D. Allison, D.L. Baho, M. Berga, H. Bürgmann, D.H. Huber, S. Langenheder, J.T. Lennon, J.B.H. Martiny, K.L. Matulich, T.M. Schmidt, J. Handelsman, Fundamentals of microbial community resistance and resilience, Front. Microbiol. 3 (2012) 1–19, https://doi.org/10.3389/fmicb.2012.00417.

[27] M. Lori, S. Symnaczik, P. Mäder, G. De Deyn, A. Gattinger, Organic farming enhances soil microbial abundance and activity — a meta-analysis and meta-regression, PLoS One 12 (2017) 1–25, https://doi.org/10.1371/journal.pone.0180442.

[28] L.B. Martínez-García, G. Korthals, L. Brussaard, H.B. Jørgensen, G.B. De Deyn, Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties, Agric. Ecosyst. Environ. 263 (2018) 7–17, https://doi.org/10.1016/j.agee.2018.04.018.

[29] H.-M. Krause, B. Stehle, J. Mayer, M. Mayer, M. Steffens, P. Mäder, A. Fliessbach, Biological soil quality and soil organic carbon change in biodynamic , organic , and conventional farming systems after 42 years, Agron. Sustain. Dev. 42 (2022) 1–14, https://doi.org/10.1007/s13593-022-00843-y.

[30] T.G. Huntington, Available water capacity and soil organic matter, Encyclopedia of Soil Science (2007) 139–143, https://doi.org/10.1081/E-ESS-120018496. Second Edition 2.

[31] K.M. Goh, Greater mitigation of climate change by organic than conventional agriculture: a review, Biological Agriculture and Horticulture 27 (2011) 205–229, https://doi.org/10.1080/01448765.2011.9756648.

[32] R.D. Bardgett, T. Caruso, Soil microbial community responses to climate extremes: resistance, resilience and transitions to alternative states. https://doi.org/10.1098/rstb.2019.0112, 2020.

[33] S. Hueso, T. Hernández, C. García, Resistance and resilience of the soil microbial biomass to severe drought in semiarid soils: the importance of organic amendments, Appl. Soil Ecol. 50 (2011) 27–36, https://doi.org/10.1016/j.apsoil.2011.07.014.

[34] S. Hueso, C. García, T. Hernández, Severe drought conditions modify the microbial community structure, size and activity in amended and unamended soils, Soil Biol. Biochem. 50 (2012) 167–173, https://doi.org/10.1016/j.soilbio.2012.03.026.
[35] E.L. Ng, A.F. Patti, M.T. Rose, C.R. Schefe, R.J. Smernik, T.R. Cavagnaro, Do organic inputs alter resistance and resilience of soil microbial community to drying? Soil Biol. Biochem. 81 (2015) 58–66, https://doi.org/10.1016/j.soilbio.2014.10.028.
[36] C. Breitkreuz, L. Herzig, F. Buscot, T. Reitz, M. Tarkka, Interactions between soil properties, agricultural management and cultivar type drive structural and functional adaptations of the wheat rhizosphere microbiome to drought, Environ. Microbiol. 23 (2021) 5866–5882, https://doi.org/10.1111/1462-2920.15607.
[37] Y. Sun, C. Tao, X. Deng, H. Liu, Z. Shen, Y. Liu, R. Li, Q. Shen, S. Geisen, Organic fertilization enhances the resistance and resilience of soil microbial communities under extreme drought, J. Adv. Res. 47 (2023) 1–12, https://doi.org/10.1016/j.jare.2022.07.009.
[38] D. Kundel, N. Bodenhausen, H.B. Jørgensen, J. Truu, K. Birkhofer, K. Hedlund, P. Mäder, A. Fliessbach, Effects of simulated drought on biological soil quality, microbial diversity and yields under long-term conventional and organic agriculture, FEMS Microbiol. Ecol. 96 (2020) 1–16, https://doi.org/10.1093/femsec/fiaa205.
[39] P. Mäder, A. Fließbach, D. Dubois, L. Gunst, P. Fried, U. Niggli, Soil fertility and biodiversity in organic farming, Science 296 (2002) 1694–1697, https://doi.org/10.1126/science.1071148, 1979.
[40] H. Azarbad, Conventional vs. organic agriculture – which one promotes better yields and microbial resilience in rapidly changing climates? Front. Microbiol. 13 (2022) 1–9, https://doi.org/10.3389/fmicb.2022.903500.
[41] C.S. Malisch, J.P. Salminen, R. Kölliker, M.T. Engström, D. Suter, B. Studer, A. Lüscher, Drought effects on proanthocyanidins in sainfoin (Onobrychis viciifolia scop.) are dependent on the plant's ontogenetic stage, J. Agric. Food Chem. 64 (2016) 9307–9316, https://doi.org/10.1021/acs.jafc.6b02342.
[42] Demeter Schweiz, Verein für biologisch-dynamische Landwirtschaft, 2019.
[43] Federal Department of Economic Affairs Education and Research, Verordnung über die Direktzahlungen an die Landwirtschaft, 2023.
[44] A.C.H. Jaeger, M. Hartmann, J. Six, E.F. Solly, Contrasting sensitivity of soil bacterial and fungal community composition to one year of water limitation in Scots pine mesocosms, FEMS Microbiol. Ecol. 99 (2023) 1–17, https://doi.org/10.1093/femsec/fiad051.
[45] Y. Liu, G.S. Duarte, Q. Sun, A.K. Gilgen, R. Wittwer, M.G.A. van der Heijden, N. Buchmann, V.H. Klaus, Severe drought rather than cropping system determines litter decomposition in arable systems, Agric. Ecosyst. Environ. 338 (2022) 1–12, https://doi.org/10.1016/j.agee.2022.108078.
[46] J. Spinoni, J.V. Vogt, G. Naumann, P. Barbosa, A. Dosio, Will drought events become more frequent and severe in Europe? Int. J. Climatol. 38 (2018) 1718–1736, https://doi.org/10.1002/joc.5291.
[47] M. Barthel, M. Bauters, S. Baumgartner, T.W. Drake, N.M. Bey, G. Bush, P. Boeckx, C.I. Botefa, N. Dériaz, G.L. Ekamba, N. Gallarotti, F.M. Mbayu, J. K. Mugula, I.A. Makelele, C.E. Mbongo, J. Mohn, J.Z. Mandea, D.M. Mpambi, L. C. Ntaboba, M.B. Rukeza, R.G.M. Spencer, L. Summerauer, B. Vanlauwe, K. Van Oost, B. Wolf, J. Six, Low N2O and variable CH4 fluxes from tropical forest soils of the Congo Basin, Nat. Commun. 13 (2022) 1–8, https://doi.org/10.1038/s41467-022-27978-6.
[48] E. Pruesse, C. Quast, K. Knittel, B.M. Fuchs, W. Ludwig, J. Peplies, F.O. Glöckner, SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB, Nucleic Acids Res. 35 (2007) 7188–7196, https://doi.org/10.1093/nar/gkm864.
[49] K. Abarenkov, R.H. Nilsson, K.-H. Larsson, I.J. Alexander, U. Eberhardt, S. Erland, K. Høiland, R. Kjøller, E. Larsson, T. Pennanen, R. Sen, A.F.S. Taylor, L. Tedersoo, B.M. Ursing, T. Vrålstad, K. Liimatainen, U. Peintner, U. Kõljalg, The UNITE database for molecular identification of fungi – recent updates and future perspectives, New Phytol. 186 (2010) 264–266, https://doi.org/10.1111/j.1469-8137.2009.03160.x.
[50] M. Longepierre, F. Widmer, T. Keller, P. Weisskopf, T. Colombi, J. Six, M. Hartmann, Limited resilience of the soil microbiome to mechanical compaction within four growing seasons of agricultural management, ISME Communications 1 (2021) 1–13, https://doi.org/10.1038/s43705-021-00046-8.
[51] S.F. Stoddard, B.J. Smith, R. Hein, B.R.K. Roller, T.M. Schmidt, rrnDB: improved tools for interpreting rRNA gene abundance in bacteria and archaea and a new foundation for future development, Nucleic Acids Res. 43 (2015) 593–598, https://doi.org/10.1093/nar/gku1201.
[52] R.B. Bledsoe, C. Goodwillie, A.L. Peralta, Long-term nutrient enrichment of an oligotroph-dominated wetland increases bacterial diversity in bulk soils and plant rhizospheres, mSphere 5 (2020) 1–12, https://doi.org/10.1128/msphere.00035-20.
[53] R Core Team, R, A Language and Environment for Statistical Computing, R foundation for statistical computing, Vienna, Austria, 2023.
[54] Posit Team, RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA, 2023.
[55] H. Wickham, M. Averick, J. Bryan, W. Chang, L. McGowan, R. François, G. Grolemund, A. Hayes, L. Henry, J. Hester, M. Kuhn, T. Pedersen, E. Miller, S. Bache, K. Müller, J. Ooms, D. Robinson, D. Seidel, V. Spinu, K. Takahashi, D. Vaughan, C. Wilke, K. Woo, H. Yutani, Welcome to the tidyverse, J. Open Source Softw. 4 (2019) 1–6, https://doi.org/10.21105/joss.01686.
[56] M.J. Anderson, A new method for non-parametric multivariate analysis of variance, Austral Ecol. 26 (2001) 32–46, https://doi.org/10.1046/j.1442-9993.2001.01070.x.
[57] M.J. Anderson, Distance-based tests for homogeneity of multivariate dispersions, Biometrics 62 (2006) 245–253, https://doi.org/10.1111/j.1541-0420.2005.00440.x.
[58] J. Oksanen, R. Kindt, P. Legendre, B.O. Hara, G.L. Simpson, P. Solymos, M. Henry, H. Stevens, H. Wagner, The vegan package:community ecology package, Community Ecology Package 10 (2008) 631–637.
[59] M. Herve, RVAideMemoire: Testing and Plotting Procedures for Biostatistics, 2023.
[60] R.A. Peterson, J.E. Cavanaugh, Ordered quantile normalization: a semiparametric transformation built for the cross-validation era, J. Appl. Stat. 47 (2020) 2312–2327, https://doi.org/10.1080/02664763.2019.1630372.
[61] R. Lenth, Emmeans: estimated marginal means, aka least-squares means, R package (2024), https://doi.org/10.1080/00031305.1980.10483031>.License version 1.10.1.
[62] P.D. Schloss, Rarefaction is currently the best approach to control for uneven sequencing effort in amplicon sequence analyses, mSphere 0 (2024) 1–20, https://doi.org/10.1128/msphere.00354-23.
[63] M.J. Anderson, T.J. Willis, Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology, Ecology 84 (2003) 511–525, https://doi.org/10.1890/0012-9658(2003)084[0511:CAOPCA]2.0.CO;2.
[64] R. Kindt, R. Coe, Tree Diversity Analysis. A Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies, World Agroforestry Centre (ICRAF), 2005.
[65] J.D. Storey, R. Tibshirani, Statistical significance for genomewide studies, Proc. Natl. Acad. Sci. USA 100 (2003) 9440–9445, https://doi.org/10.1073/pnas.1530509100.
[66] I. Letunic, P. Bork, Interactive Tree of Life v2: online annotation and display of phylogenetic trees made easy, Nucleic Acids Res. 39 (2011) 475–478, https://doi.org/10.1093/nar/gkr201.
[67] S. Dray, A.B. Dufour, The ade4 package: implementing the duality diagram for ecologists, J. Stat. Software 22 (2007) 1–20, https://doi.org/10.18637/jss.v022.i04.
[68] W.J. Rawls, Y.A. Pachepsky, J.C. Ritchie, T.M. Sobecki, H. Bloodworth, Effect of soil organic carbon on soil water retention, Geoderma 116 (2003) 61–76, https://doi.org/10.1016/S0016-7061(03)00094-6.
[69] B. Minasny, A.B. McBratney, Limited effect of organic matter on soil available water capacity, Eur. J. Soil Sci. 69 (2018) 39–47, https://doi.org/10.1111/ejss.12475.
[70] A. Gattinger, A. Muller, M. Haeni, C. Skinner, A. Fliessbach, N. Buchmann, P. Mäder, M. Stolze, P. Smith, N.E.H. Scialabba, U. Niggli, Enhanced top soil carbon stocks under organic farming, Proc. Natl. Acad. Sci. USA 109 (2012) 18226–18231, https://doi.org/10.1073/pnas.1209429109.
[71] A. Dai, Increasing drought under global warming in observations and models, Nat. Clim. Change 3 (2013) 52–58, https://doi.org/10.1038/nclimate1633.
[72] M. Zhao, J. Zhao, J. Yuan, L. Hale, T. Wen, Q. Huang, J.M. Vivanco, J. Zhou, G. A. Kowalchuk, Q. Shen, Root exudates drive soil-microbe-nutrient feedbacks in response to plant growth, Plant Cell Environ. 44 (2021) 613–628, https://doi.org/10.1111/pce.13928.
[73] P. Marschner, G. Neumann, A. Kania, L. Weiskopf, R. Lieberei, Spatial and temporal dynamics of the microbial community structure in the rhizosphere of cluster roots of white lupin (Lupinus albus L.), Plant Soil 246 (2002) 167–174, https://doi.org/10.1023/A:1020663909890.
[74] C.L. Lauber, K.S. Ramirez, Z. Aanderud, J. Lennon, N. Fierer, Temporal variability in soil microbial communities across land-use types, ISME J. 7 (2013) 1641–1650, https://doi.org/10.1038/ismej.2013.50.
[75] K. Khadka, H.J. Earl, M.N. Raizada, A. Navabi, A physio-morphological trait-based approach for breeding drought tolerant wheat, Front. Plant Sci. 11 (2020) 1–26, https://doi.org/10.3389/fpls.2020.00715.
[76] R.A. Wittwer, V.H. Klaus, E. Miranda Oliveira, Q. Sun, Y. Liu, A.K. Gilgen, N. Buchmann, M.G.A. van der Heijden, Limited capability of organic farming and conservation tillage to enhance agroecosystem resilience to severe drought, Agric. Syst. 211 (2023) 1–11, https://doi.org/10.1016/j.agsy.2023.103721.
[77] K.M. Murphy, K.G. Campbell, S.R. Lyon, S.S. Jones, Evidence of varietal adaptation to organic farming systems, Field Crops Res. 102 (2007) 172–177, https://doi.org/10.1016/j.fcr.2007.03.011.
[78] M.C. Oguz, M. Aycan, E. Oguz, I. Poyraz, M. Yildiz, Drought stress tolerance in plants: interplay of molecular, biochemical and physiological responses in important development stages, Physiol. 2 (2022) 180–197, https://doi.org/10.3390/physiologia2040015.
[79] E. Ciríaco Da Silva, R. Mansur Jurema Custódio Nogueira, M. Almeida Da Silva, M. Bandeira De Albuquerque, Drought stress and plant nutrition, Plant Stress 5 (2009) 32–41.
[80] A. Bhattacharya, Soil Water Deficit and Physiological Issues in Plants, Springer Singapore, 2021, https://doi.org/10.1007/978-981-33-6276-5.
[81] M. Tränkner, B. Jákli, E. Tavakol, C.M. Geilfus, I. Cakmak, K. Dittert, M. Senbayram, Magnesium deficiency decreases biomass water-use efficiency and increases leaf water-use efficiency and oxidative stress in barley plants, Plant Soil 406 (2016) 409–423, https://doi.org/10.1007/s11104-016-2886-1.
[82] L. Fuchslueger, M. Bahn, K. Fritz, R. Hasibeder, A. Richter, Experimental drought reduces the transfer of recently fixed plant carbon to soil microbes and alters the bacterial community composition in a mountain meadow, New Phytol. 201 (2014) 916–927, https://doi.org/10.1111/nph.12569.
[83] A. Canarini, Y. Carrillo, P. Mariotte, L. Ingram, F.A. Dijkstra, Soil microbial community resistance to drought and links to C stabilization in an Australian grassland, Soil Biol. Biochem. 103 (2016) 171–180, https://doi.org/10.1016/j.soilbio.2016.08.024.

[84] P. Carini, P.J. Marsden, J.W. Leff, E.E. Morgan, M.S. Strickland, N. Fierer, Relic DNA is abundant in soil and obscures estimates of soil microbial diversity, Nat Microbiol 2 (2016), https://doi.org/10.1038/nmicrobiol.2016.242.
[85] M. Hartmann, I. Brunner, F. Hagedorn, R.D. Bardgett, B. Stierli, C. Herzog, X. Chen, A. Zingg, E. Graf-Pannatier, A. Rigling, B. Frey, A decade of irrigation transforms the soil microbiome of a semi-arid pine forest, Mol. Ecol. 26 (2017) 1190–1206, https://doi.org/10.1111/mec.13995.
[86] J. Li, G. Benti, D. Wang, Z. Yang, R. Xiao, Effect of alteration in precipitation amount on soil microbial community in a semi-arid grassland, Front. Microbiol. 13 (2022) 1–7, https://doi.org/10.3389/fmicb.2022.842446.
[87] D.V. Sapronov, Y.V. Kuzyakov, Separation of root and microbial respiration: comparison of three methods, Eurasian Soil Sci. 40 (2007) 775–784, https://doi.org/10.1134/S1064229307070101.
[88] J. Hirte, F. Walder, J. Hess, L. Büchi, T. Colombi, M.G. van der Heijden, J. Mayer, Enhanced root carbon allocation through organic farming is restricted to topsoils, Sci. Total Environ. 755 (2021) 143–551, https://doi.org/10.1016/j.scitotenv.2020.143551.
[89] T. Hu, P. Sørensen, E.M. Wahlström, N. Chirinda, B. Sharif, X. Li, J.E. Olesen, Root biomass in cereals, catch crops and weeds can be reliably estimated without considering aboveground biomass, Agric. Ecosyst. Environ. 251 (2018) 141–148, https://doi.org/10.1016/j.agee.2017.09.024.
[90] S.D. Frey, E.T. Elliott, K. Paustian, Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients, Soil Biol. Biochem. 31 (1999) 573–585, https://doi.org/10.1016/S0038-0717(98)00161-8.
[91] H.A. Gamper, J.P.W. Young, D.L. Jones, A. Hodge, Real-time PCR and microscopy: are the two methods measuring the same unit of arbuscular mycorrhizal fungal abundance? Fungal Genet. Biol. 45 (2008) 581–596, https://doi.org/10.1016/j.fgb.2007.09.007.
[92] J. Fang, S. Wei, Y. Gao, X. Zhang, Y. Cheng, J. Wang, J. Ma, G. Shi, L. Bai, R. Xie, X. Zhao, Y. Ren, Z. Lu, Character variation of root space microbial community composition in the response of drought-tolerant spring wheat to drought stress, Front. Microbiol. 14 (2023) 1–15, https://doi.org/10.3389/fmicb.2023.1235708.
[93] K.E. Bazany, J.T. Wang, M. Delgado-Baquerizo, B.K. Singh, P. Trivedi, Water deficit affects inter-kingdom microbial connections in plant rhizosphere, Environ. Microbiol. 24 (2022) 3722–3734, https://doi.org/10.1111/1462-2920.16031.
[94] W.J. Landesman, A.M. Treonis, J. Dighton, Effects of a one-year rainfall manipulation on soil nematode abundances and community composition, Pedobiologia 54 (2011) 87–91, https://doi.org/10.1016/j.pedobi.2010.10.002.
[95] S. Geisen, C. Bandow, J. Römbke, M. Bonkowski, Soil water availability strongly alters the community composition of soil protists, Pedobiologia 57 (2014) 205–213, https://doi.org/10.1016/j.pedobi.2014.10.001.
[96] F.T. De Vries, A. Shade, Controls on soil microbial community stability under climate change, Front. Microbiol. 4 (2013) 1–16, https://doi.org/10.3389/fmicb.2013.00265.
[97] K. Hartman, S.G. Tringe, Interactions between plants and soil shaping the root microbiome under abiotic stress, Biochem. J. 476 (2019) 2705–2724, https://doi.org/10.1042/BCJ20180615.
[98] L.H. Comas, S.R. Becker, V.M.V. Cruz, P.F. Byrne, D.A. Dierig, Root traits contributing to plant productivity under drought, Front. Plant Sci. 4 (2013) 1–16, https://doi.org/10.3389/fpls.2013.00442.
[99] A. Carminati, A.B. Moradi, D. Vetterlein, P. Vontobel, E. Lehmann, U. Weller, H. J. Vogel, S.E. Oswald, Dynamics of soil water content in the rhizosphere, Plant Soil 332 (2010) 163–176, https://doi.org/10.1007/s11104-010-0283-8.
[100] R. Serraj, T.R. Sinclair, Osmolyte accumulation: can it really help increase crop yield under drought conditions? Plant Cell Environ. 25 (2002) 333–341, https://doi.org/10.1046/j.1365-3040.2002.00754.x.
[101] M. Sünnemann, J. Siebert, T. Reitz, M. Schädler, R. Yin, N. Eisenhauer, Combined effects of land-use type and climate change on soil microbial activity and invertebrate decomposer activity, Agric. Ecosyst. Environ. 318 (2021) 1–8, https://doi.org/10.1016/j.agee.2021.107490.
[102] K. Birkhofer, A. Fliessbach, M.P. Gavín-Centol, K. Hedlund, M. Ingimarsdóttir, H. B. Jørgensen, K. Kozjek, S. Meyer, M. Montserrat, S.S. Moreno, J.M. Laraño, S. Scheu, D. Serrano-Carnero, J. Truu, D. Kundel, Conventional agriculture and not drought alters relationships between soil biota and functions, Sci. Rep. 11 (2021) 1–12, https://doi.org/10.1038/s41598-021-03276-x.
[103] P. Li, L. Tedersoo, T.W. Crowther, A.J. Dumbrell, F. Dini-Andreote, M. Bahram, L. Kuang, T. Li, M. Wu, Y. Jiang, L. Luan, M. Saleem, F.T. de Vries, Z. Li, B. Wang, J. Jiang, Fossil-fuel-dependent scenarios could lead to a significant decline of global plant-beneficial bacteria abundance in soils by 2100, Nat Food 4 (2023) 996–1006, https://doi.org/10.1038/s43016-023-00869-9.
[104] S. Saini, I. Sharma, N. Kaur, P.K. Pati, Auxin: a master regulator in plant root development, Plant Cell Rep. 32 (2013) 741–757, https://doi.org/10.1007/s00299-013-1430-5.
[105] B.R. Glick, B. Todorovic, J. Czarny, Z. Cheng, J. Duan, B. McConkey, Promotion of plant growth by bacterial ACC deaminase, CRC Crit. Rev. Plant Sci. 26 (2007) 227–242, https://doi.org/10.1080/07352680701572966.
[106] R. Saha, N. Saha, R.S. Donofrio, L.L. Bestervelt, Microbial siderophores: a mini review, J. Basic Microbiol. 53 (2013) 303–317, https://doi.org/10.1002/jobm.201100552.
[107] C. Santos-Medellín, Z. Liechty, J. Edwards, B. Nguyen, B. Huang, B.C. Weimer, V. Sundaresan, Prolonged drought imparts lasting compositional changes to the rice root microbiome, Nat. Plants 7 (2021) 1065–1077, https://doi.org/10.1038/s41477-021-00967-1.
[108] B. Sahoo, R. Ningthoujam, S. Chaudhuri, Isolation and characterization of a lindane degrading bacteria Paracoccus sp. NITDBR1 and evaluation of its plant growth promoting traits, Int. Microbiol. 22 (2019) 155–167, https://doi.org/10.1007/s10123-018-00037-1.
[109] Y.N. Kim, M.A. Khan, S.M. Kang, M. Hamayun, I.J. Lee, Enhancement of drought-stress tolerance of brassica oleracea var. italica L. By newly isolated variovorax sp. YNA59, J. Microbiol. Biotechnol. 30 (2020) 1500–1509, https://doi.org/10.4014/JMB.2006.06010.
[110] S. Tistechok, M. Skvortsova, Y. Mytsyk, V. Fedorenko, I. Parnikoza, A. Luzhetskyy, O. Gromyko, The diversity and antibacterial activity of culturable actinobacteria isolated from the rhizosphere soil of Deschampsia Antarctica (Galindez Island, Maritime Antarctic), Polar Biol. 44 (2021) 1859–1868, https://doi.org/10.1007/s00300-021-02924-2.
[111] A. Chauhan, P.P. Balgir, C.K. Shirkot, Characterization of Aneurinibacillus aneurinilyticus strain CKMV1 as a plant growth promoting rhizobacteria, Int. J. Agric. Environ. Biotechnol. 7 (2014) 37–45, https://doi.org/10.5958/j.2230-732x.7.1.006.
[112] G. Cuesta, R. García-de-la-Fuente, M. Abad, F. Fornes, Isolation and identification of actinomycetes from a compost-amended soil with potential as biocontrol agents, J. Environ. Manag. 95 (2012) 280–284, https://doi.org/10.1016/j.jenvman.2010.11.023.
[113] Z. Zhang, C. Henderson, E. Perfect, T.L.W. Carver, B.J. Thomas, P. Skamnioti, S. J. Gurr, Of genes and genomes, needles and haystacks: Blumeria graminis and functionality, Mol. Plant Pathol. 6 (2005) 561–575, https://doi.org/10.1111/j.1364-3703.2005.00303.x.
[114] H.T. Doğmuş-Lehtijärvi, A. Lehtijärvi, S. Woodward, F. Oskay, Impacts of inoculation with Herpotrichia pinetorum, Gremmenia infestans and Gremmeniella abietina on Pinus nigra subsp. pallasiana and Cedrus libani seedlings in the field, For. Pathol. 46 (2016) 47–53, https://doi.org/10.1111/efp.12213.
[115] R. Aroca, M. Del Mar Alguacil, P. Vernieri, J.M. Ruiz-Lozano, Plant responses to drought stress and exogenous ABA application are modulated differently by mycorrhization in tomato and an ABA-deficient mutant (Sitiens), Microb. Ecol. 56 (2008) 704–719, https://doi.org/10.1007/s00248-008-9390-y.
[116] M.S. Goettel, M. Koike, J.J. Kim, D. Aiuchi, R. Shinya, J. Brodeur, Potential of Lecanicillium spp. for management of insects, nematodes and plant diseases, J. Invertebr. Pathol. 98 (2008) 256–261, https://doi.org/10.1016/j.jip.2008.01.009.
[117] H. Baltruschat, V.M. Santos, D.K.A. da Silva, I. Schellenberg, A. Deubel, E. Sieverding, F. Oehl, Unexpectedly high diversity of arbuscular mycorrhizal fungi in fertile Chernozem croplands in Central Europe, Catena 182 (2019) 1–11, https://doi.org/10.1016/j.catena.2019.104135.
[118] E.L.M. De Almeida, R.Z. Ventorim, M.A. De Moura Ferreira, W.B. Da Silveira, Papiliotrema laurentii: general features and biotechnological applications, Appl. Microbiol. Biotechnol. 106 (2022) 6963–6976, https://doi.org/10.1007/s00253-022-12208-2.
[119] E. Labancová, K. Šípošová, D. Kučerová, Á. Horváthová, H. Schusterová, Z. Vivodová, R. Vadkertiová, K. Kollárová, The tremellaceous yeast: Papiliotrema terrestris—as the growth stimulant of maize plants, J. Plant Growth Regul. 42 (2023) 3835–3850, https://doi.org/10.1007/s00344-022-10848-2.
[120] F.A. Rainey, Trichococcus , Bergey's Manual of Systematics of Archaea and Bacteria, 2015, pp. 1–7, https://doi.org/10.1002/9781118960608.gbm00599.
[121] S. Chen, Saccharofermentans, Bergey's Manual of Systematics of Archaea and Bacteria, 2017, pp. 1–5, https://doi.org/10.1002/9781118960608.gbm01450.
[122] Y. Kong, R. Teather, R. Forster, Composition, spatial distribution, and diversity of the bacterial communities in the rumen of cows fed different forages, FEMS Microbiol. Ecol. 74 (2010) 612–622, https://doi.org/10.1111/j.1574-6941.2010.00977.x.
[123] Y. Bi, S. Zeng, R. Zhang, Q. Diao, Y. Tu, Effects of dietary energy levels on rumen bacterial community composition in Holstein heifers under the same forage to concentrate ratio condition, BMC Microbiol. 18 (2018) 1–11, https://doi.org/10.1186/s12866-018-1213-9.